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[*Authors alone are responsible for all opinions expressed in their Communications.*]

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No. 42.

# ON THE COMPARISON OF THE GAMMA RAY INTENSITIES FROM RADIUM PREPARATIONS.

By J. A. C. TEEGAN, M.Sc., A. Inst. P.

[Read June 23. Published separately December 16, 1936.]

It is frequently necessary in radioactive work to measure accurately the quantity of radium present in a preparation of a radium salt. The method usually employed depends on the fact that the radium in a salt sealed in a tube, so that no emanation can escape, is, after a period of a month in equilibrium with its short-lived products, and will, therefore, emit $\gamma$-rays of intensity proportional to the amount of radium present. The intensity of $\gamma$-ray emission is usually measured by the simple electroscope, or by a combination of electrometer and ionization chamber. In either case the walls of the vessel in which the ionization is produced should be thick enough to completely absorb the $\beta$-rays simultaneously emitted from the preparation, and a sufficiently high voltage applied to the ionization chamber to produce saturation. Under these circumstances the ionization current produced is a measure of the $\gamma$-ray intensity.

A compensation method of measurement, in which an accuracy of 1 in 400 is claimed, has been described by Rutherford and Chadwick (1).

The development of the electroscope methods has been very fully described by Bastings (2), who enumerates the various factors which tend to limit the accuracy of the measurements.

The chief sources of inaccuracy associated with the electroscope method are :—

(1) Variations in natural leak of the instrument.
(2) Instrumental and personal errors in timing the movement of the leaf.
(3) Effect of convection due to heat variations.
(4) Difficulty of controlling the sensitivity.
(5) Time taken in making a measurement.

A steady deflection (or null) method of measuring $\gamma$-ray intensity is described in this paper, in which the disadvantages of the electroscope method outlined above are eliminated, and a high degree of accuracy is attainable without any special refinements.

It is a development of a method of ionization measurement previously described by the author (3), and consists in the amplification of the ionization

produced by the $\gamma$-rays emitted from radium needles placed outside a specially constructed ionization chamber.

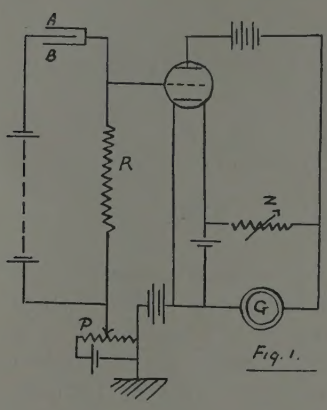

Fig. 1.

The amplifier consists of an electrometer valve unit (see figure) completely enclosed in an earth-shielded box fitted with an ebonite panel for the controls. $R$ is a high resistance leak, composed of a mixture of xylol and alcohol contained in a sealed tube. $AB$ is the ionization chamber, which may also be mounted within the box.

On ionizing the air, or other gas within the chamber, a current $C$ flows through the leak to earth, thereby producing a potential $CR$ on the grid, and a resulting deflection $SmCR$ of the galvanometer in the anode circuit, $m$ being the mutual conductance of the valve, and $S$ the sensitivity of the galvanometer.

Observations were made with a number of radium preparations ranging from 2–12 mg. Using a cylindrical chamber 11 cm. long and 4 cm. diameter with walls of lead 3 mm. thick, the preparations were placed successively in a small slot mounted on one end of the chamber, and readings were obtained—first with air in the chamber, and then for a number of different gases.

The results for the different gases show that the absorption increases with the number of electrons in the molecule of the absorbing gas.

If the absorption were a pure $\gamma$-ray (Compton) scattering effect, the ionization should increase linearly with the number of electrons in the scattering molecules. The departure from linearity of the relation between ionization current and number of electrons per molecule may be attributed to the fact that the ionization in the chamber is produced partly by the $\gamma$-rays which pass through the walls of the chamber without absorption and partly by the $\beta$-rays produced in the lead from the $\gamma$-rays.

It is evident from the table that in all cases, except that of methyl bromide, the ionization is proportional to the strength of the radium preparation. The want of regularity in the case of methyl bromide is due to the fact that the increase in the anode current is so large that the bending of the valve characteristic becomes of importance.

Errors due to the curvature of the valve characteristic are of importance in accurate work, also when the instrument is used for making measurements over a wide range of specimens. For this reason the "null" method is preferable, in which a potentiometer $P$ is inserted in the grid circuit. On inserting a specimen the grid potential is restored to its original value by alteration of the potentiometer $P$, the reading of the potentiometer being proportional to the amount of radium present.

Fluctuations in the valve emission make it impossible to standardize the

readings of the potentiometer in terms of milligrams of radium, and it is advisable to calibrate the instrument fairly regularly. A range of specimens from (0·25–12) mg. of radium have been measured by this method.

This covers the range of measurement required for ordinary hospital routine work, for which purpose the method would appear to be more suitable than the ordinary γ-ray electroscope, in respect of the rapidity with which measurements can be made, and the greater certainty associated with steady deflection or "null" methods.

A special feature of this method is its freedom from irregularities associated with natural leak, which is usually very large in buildings used for radiological purposes, as any natural leak in the chamber is balanced out initially by variation of the compensating resistance $Z$ in the anode circuit.

The instrument has been built up in a small cabinet, fitted with an ebonite panel, on which the galvanometer and terminals are mounted. The cabinet is lined with sheet tin (earth connected) to shield the triode from external disturbances. Ebonite is a sufficiently good insulator for all parts of the circuit, except that a collar of amber or sulphur is used for mounting the terminal connecting the grid to the ionization chamber.

The compensating variable resistance in the anode circuit is of the "slider type," as used for wireless purposes; it has a total resistance of 25,000 ohms.

The sensitivity can be increased in a number of ways: (1) by increasing the grid leak $R$, (2) increasing the size of the ionization chamber, (3) using a more sensitive galvanometer, (4) employing a heavy gas in the chamber.

TABLE.

*Galvanometer Deflection.*

| Milligrams of Radium. | Air | Oxygen | $CO_2$ | $SO_2$ | $CH_3Br$. |
|---|---|---|---|---|---|
| 2 | 9·5 | 10 | 12 | 16 | 28 |
| 5 | 22 | 22 | 28 | 40 | 62 |
| 7 | 34 | 36 | 42 | 56 | 90 |
| 10 | 50 | 52 | 64 | 80 | 120 |
| 12 | 60 | 62 | 76 | 94 | 146 |
| 2 | 84 | — | 101 | 123 | 210 |
| 5 | 200 | — | 252 | 315 | 509 |

Increase of grid leak resistance is limited if rapid measurements are required, and this should not exceed $10^{12}$ ohms. The readings recorded in the table were obtained with a grid leak of the order $10^{11}$ ohms; the sensitivity can obviously be increased about tenfold by using a leak of $10^{12}$ ohms. Data for the construction of these high resistances are given by Campbell (4), and a convenient method of approximately estimating their value has been described by the author (5). The readings in the upper part of the table were obtained with a galvanometer of sensitivity 2 divs. per microampere, those in the lower part with a galvanometer of sensitivity 20 mm. per micro-amp. The latter were obtained under very steady conditions. The increase of sensitivity by the use of sensitive galvano-

meters is, however, limited by fluctuations in the anode and filament supply batteries, and it is preferable to use any of the other methods available for obtaining high sensitivity.

Measurements have been made when an X-ray installation was in operation in the room, and no unsteadiness was apparent in the absence of excessive "brush" discharging. This freedom from external electromagnetic effects is presumably due to excessive damping in the grid circuit, which contains very high resistance, and relatively little inductance or capacity.

The author wishes to thank Professor W. L. Bragg, F.R.S., and Dr. J. M. Nuttall for their kind interest in the work; also the Board of the John Harling Fellowship for a grant which made the work possible.

The radium was kindly lent by Dr. Paterson, of the Manchester Radium Institute.

Physical Laboratories,

University, Manchester, *June*, 1933.

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No. 43.

# AWARD OF THE BOYLE MEDAL TO DR. H. H. POOLE.

1936.[1]

REPORT OF THE COMMITTEE OF SCIENCE AND ITS INDUSTRIAL APPLICATIONS.

DR. POOLE'S scientific publications began with a notable series of papers upon the physical properties of rocks and minerals at high temperatures, chiefly in connection with the late Professor Joly's geophysical theories. Starting in 1910 this series includes studies on radioactive heat evolution, thermal conductivity, dielectric constant, electrical conductivity and its temperature coefficient, also papers on tidal stresses and continental displacements, sub-continental temperatures and thermal instability of the earth's crust. These are characterised by accurate work and experimental ingenuity, combined with a highly critical attitude of mind. The work on the electrical properties of dielectrics in very strong electric fields, and at different temperatures, proved that for large potential gradients a dielectric does not obey Ohm's law, and he deduced an empirical law for the connection between current and voltage in such cases. This work has received a good deal of attention in Germany, where much investigation on the same lines has been conducted since. He also developed a very neat vector method of dealing with various induction and capacity measurements.

He has also applied his knowledge and experimental skill as a physicist to biological problems in collaboration with other members of the Society. This work includes the testing of a photo-electric theory of photo-synthesis, and the measurement of the penetration of light of various wave-lengths into the sea. The method used for the latter, published in the Society's Proceedings in 1925 in his sole name, has proved of great value, and has enabled such measurements to be made around the coasts of Europe and America and right across the Atlantic. He has also collaborated in work upon the illumination in woods, upon the standardization of photo-electric cells for the measurement of daylight and upon the seasonal changes in daylight.

The work already carried out by Dr. H. H. Poole gives us reason to hope that he may continue in the future to assist stoutly in upholding the Society's position in the learned world.

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[1] The medal was awarded by the Council of the Royal Dublin Society on February 13th, 1936, and was presented to Dr. Poole at a special meeting on March 26th, 1936, by Mr. Harry Franks, M.A., F.S.I., President of the Society.

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No. 44.

# FERTILIZATION AND PROEMBRYO FORMATION IN *SEQUOIA*.

By W. J. LOOBY

AND

J. DOYLE.

(Botany Department, University College, Dublin.)

(Plates 13 and 14.)

[Read May 28, 1935. Published separately January 11, 1937.]

Development in *Sequoia*, which is here taken to include the two species *S. gigantea* Decaisne and *S. sempervirens* Endl., has been studied by Shaw (12), Arnoldi (1, 2, 3), and Lawson (6). These authors have mainly dealt with *S. sempervirens*, Arnoldi (3) alone referring to some important points in the gametophytes of *S. gigantea*; but it is clear from their work that *S. sempervirens* is a conifer of peculiar interest. The development of several gynospores, supernumerary prothalli, lateral pollen tubes and lateral archegonia; the manner in which most of the male cell cytoplasm remains outside the archegonium at fertilization; especially wall formation at the first post-fertilization division, all seem to give the redwood an unique position. It is therefore remarkable that, except for the observations of Arnoldi on the prothallus of *S. gigantea*, no adequate study has been made of this species. Apparently there has been a tacit assumption that the details of the life-history of the big-tree are similar to those of *S. sempervirens* except, perhaps, for a possible difference in the prothallial development, a difference which has never been confirmed. This work was begun with the purpose of testing this assumption in the case of *S. gigantea* and, further, of clarifying, if possible, certain points in the development of *S. sempervirens*.

## A. *Sequoia gigantea*.

The obvious necessity of completing the life-history of *S. gigantea* has been under consideration for some time, but the work was delayed by an apparent difficulty. Although in the gardens and estates in Dublin and Wicklow there are many fine coning specimens of this tree, examination of mature cones showed a very low percentage of developed seed. With so low a degree of fertility the chances of securing critical stages in the life-history seemed too remote to make the labour involved worth while. Examination, however, of a chance collection of young cones made in the latter half of August in a recent year showed, surprisingly, a very high degree of fertility. Pollen tubes, embryo

stages and the like were obtainable in most of the ovules cut. It was soon clear that pollination, fertilization, and proembryo development, are carried out with at least normal, frequently with very considerable, success in the first year. The winter after pollination is passed in an advanced condition of cleavage polyembryony, masses of coiled suspensors with attached embryo cells being readily dissected from most of the ovules, but, with the advance of the second year, a very high embryonic mortality ensues, for reasons at present unknown, resulting in the mature condition of only an occasional good seed in the older cones. This embryo mortality seems a common phenomenon in these islands, and may be associated with the obvious point that all specimens growing here are still, for so long-lived a species, quite young. To what extent it occurs in young trees growing under natural conditions in California is unknown to us.

The present account is limited to the mature gametophytes, fertilization, and proembryo formation. Arnoldi does not deal with fertilization and later phenomena, nor is there in his work or elsewhere any account of the earliest stages in gynospore origin and development except a passing remark by him that more than one prothallus can be seen in very young ovules, suggesting the possible presence of more than one gynospore. Arnoldi, however, as already mentioned, emphasizes a difference in prothallial development between *S. gigantea* and *S. sempervirens.* As it is hoped to deal in a later paper with the earlier stages in the development of the prothallus in *S. gigantea,* and the difference, if any, in the gametophyte development in the two species, the point need not be further considered here.

*Gametophytes and fertilization.*

Ovules collected about the third week of August in these latitudes should show stages approaching fertilization. Arnoldi has already recorded the fact that in general the ovular structure resembles that of *S. sempervirens.* Pollen tubes have grown down laterally along the sides of the female prothallus, the tubes being of a typical cupressinean type with two equal male cells. Archegonia are developed, also laterally, more or less in a complex in connection with the functioning portion of the tube, and scattered elsewhere. These observations are correct, but they can be supplemented with so many points of detail that it is perhaps better to describe the ovular structure at maturity without further regard to Arnoldi's rather limited account.

A diagrammatic longitudinal section of a fertile ovule is shown in Fig. 1. As the ovule, owing to local pressure, is frequently a little curved, and as the pollen tube is never straight, it is difficult to secure a perfect median section. Fig. 1 is therefore to some extent of a composite nature. The pollen tube penetrates the nucellus early, and is found for most of its length lying between the nucellus and the gynospore membrane. At a certain distance down it shows a hemispherical expansion on one side, which invaginates the female prothallus. In this expansion the body cell or male cells will be found according to the stage of development. It then continues further down, showing the same more

or less tubular form which it has above the expansion, finally tapering abruptly to its end. One of the sections from which the diagram was taken is photographed on Pl. 13: 1. This "tail" to the pollen tube is not recorded for the

FIG. 1.—*Sequoia gigantea.* L.S. of ovule about fertilization (diagrammatic) showing the general arrangement of the structures, especially the path and shape of the pollen tube, the position of the functioning archegonia in a rough complex, and the distinct gynospore membrane (the heavy line) surrounding the prothallus. × 20. Aug. 24.

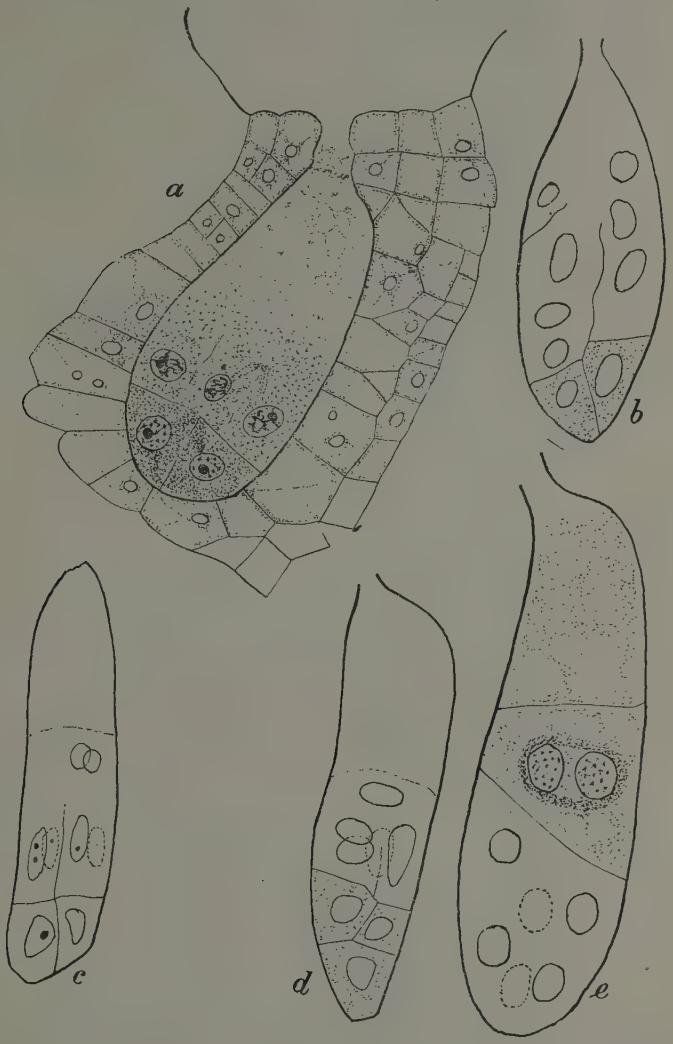


FIG. 2.—*Sequoia gigantea.* *a.* Typical 8-celled proembryo in section, with surrounding prothallial cells, showing a 2 : 6 arrangement. *b, c, d, e.* Four examples (diagrammatic) of abnormal young proembryos all 8-nucleate except *b* in which one nucleus has divided further; the upper dotted line in *c* and *d* represents the visible limit of denser proembryonic cytoplasm, the thin continuous lines in each case representing walls. All × 330. Aug. 24.

*Callitrineae*, the other group of conifers with lateral pollen tubes and archegonia, except for a short extension in *Callitris* (9). It seems definitely absent in *Widdringtonia* (7, 8) and *Actinostrobus* (10). The expansion of the tube which so markedly invaginates the prothallus in *Sequoia* seems also absent in the callitrinean genera. The pollen tube wall is quite firm in its whole length, permitting considerable manipulation, so that preparations of various stages of the cupressinean type of male gametophyte can be readily dissected out. The nucellus at this stage is still a very definite tissue several cells thick. As the nucellus in *Sequoia sempervirens* is very much more reduced, except at the apex, this difference permits of a ready distinguishing between transverse sections of ovules of the two species at this stage. It is shown for example by Pl. 13: 2 (*S. gigantea*) and Pl. 14: 18 (*S. sempervirens*). The long narrow female gametophyte is very different in its upper and lower parts. The lower part, obviously of later development, is composed of fairly dense irregularly dividing cells forming a firm compact tissue, the cells of the upper part being much less compact and frequently regularly arranged as if formed from original alveoli. The archegonia are arranged in an irregular complex in contact with the pollen tube expansion and are scattered above it, but usually in one or more vertical rows in contact with, or directed towards, the upper part of the pollen tubes. It is hoped to publish later a detailed account of the development of all this tissue together with that of *Sequoia sempervirens* and the species of *Athrotaxis*, as there are many important aspects of these stages still obscure. No further reference to them will be made here.

The functional archegonia are normally those in contact with the expansion of the pollen tube. These are not numerous, usually not more than twenty or so, nor can they really be said to lie in a true complex. They are scattered irregularly over the hemispherical invaginated area with a strong tendency to the lower half. Here, if numerous, a number may be in contact looking in transverse section like a real cupressinean complex, while a few sections higher a zone may occur with no archegonia, or with archegonia separated by regular rows of small cells. There may be an abrupt transition between the group of functional archegonia and the commonly less normal and more scattered ones higher up, or such a transition may be gradual. If more than one tube is present there will of course be a corresponding number of these archegonial groups associated with each pollen tube intrusion. There are only traces of jacket-like cells. An examination of the sections photographed for the plates shows the occasional presence of slightly larger and denser cells with more conspicuous nuclei, but their sparse occurrence, even where the archegonia are in a complex-like group, and their general indefiniteness are very apparent. On the whole, as already mentioned, the archegonia tend to develop towards the pollen tube, but that the presence of the tube is not the only factor determining their development is shown by the fairly frequent occurrence of normally mature archegonia on the periphery with necks in contact with the gynospore membrane elsewhere than at the pollen tube. This is much less common here than in *Sequoia sempervirens*.

The mature archegonia are of the cupressinean type—small, with fairly uniform protoplasm and a large vacuole at the lower part, which gradually decreases with approaching maturity. The peculiar protoplasmic areas described in the archegonia of other cupressinean conifers (cf. 4 for references) are either absent or very poorly developed. The division to give a ventral canal nucleus was not seen. The ventral canal nucleus is apparently rather ephemeral, but occasionally remains and may become rather prominent. There are, however, some peculiarities which cannot yet be fully discussed as they need further elucidation. The neck-cells are most commonly arranged in one tier of four (Pl. 13: 3), although Arnoldi gives definitely two as the number. The second division may sometimes not take place, but four seems to be the characteristic number, although here again occur some interesting peculiarities which are still being examined. The neck-cells persist till after fertilization. The gynospore membrane is quite thick for a conifer of this affinity, and is usually very conspicuous, especially in transverse sections. Such a section is shown in Pl. 13: 2. It is taken just below the tube expansions and through the lower extensions. It is very clear here that the membrane is evenly intact all the way round, the pollen tube extensions being completely outside. A section above the tube expansion would show a similar condition, except that, the whole gametophytic tissue being here somewhat more delicate, the membrane would appear a little less definite, though the difference is not very great. At the invagination, however, as shown in Pl. 13: 4, the thick membrane can be seen turning in and gradually thinning off over the functioning archegonia. The technique so far employed in the preparation of the material was not sufficiently delicate to enable a distinction to be made between the gynospore membrane and the pollen tube wall over the whole surface of the invagination. The membrane, however, appears to be thinnest—if retained at all at this stage—over the necks of the lower archegonia of the group, the archegonia which tend to form the more definite complex, and which later are most commonly fertilized. On the upper area of the surface of the invagination the membrane may be distinctly visible. It is clear, at any rate, that the tube is wholly outside the membrane, and that the functional archegonia at maturity are in contact with it at the periphery, or at least with the plane it would occupy if indeed it has completely vanished over a small area. This point will also arise later in the examination of the development stages, as the position of origin of archegonial initials, whether deep-seated or peripheral, is still in doubt in some cases, particularly perhaps in the species of *Sequoia* and *Athrotaxis*.

In slightly younger stages the pollen tube shows the usual content—two sterile nuclei and one body cell. The sterile nuclei may be seen for some time but have usually disappeared before fertilization, otherwise they call for no comment. The body cell is rather ellipsoid in shape, and is shown cut in its long axis in Pl. 13: 4, and cut in its short axis in Pl. 13: 5. The nucleus is large and spherical, the nuclear membrane being usually very distinct. The main mass of the cytoplasm is uniform and fairly dense, but a narrow zone, like the ectoplasm of an *Amœba*, of more transparent and less granular proto-

plasm commonly is seen at the periphery which is finally limited by a rather delicate membrane. An intranuclear mitosis, seen though not figured, initiates the division of the body cell into two hemispherical parts which soon separate and round off as more or less spherical male cells. The appearance when so just rounded off is shown in Pl. 13 : 6. The nucleus is small, and the main mass of the cytoplasm is still dense, but the peripheral clearer zone is much more distinct than in the body cell. This section also shows a peculiarity which is very common, appearing sometimes even before the body cell division (Pl. 13 : 4), and found just up to fertilization. It can be seen that the neck and upper part of an archegonium are projecting beyond the level of the surrounding tissue, as if stretching out towards the male cell. It is not safe to say that it is always to be seen, it is certainly not to be seen in all the archegonia of the group surrounding the invagination, and it is for the present only suggested that when clearly seen, as here, it represents an archegonium already related in some special way to, and marked for, fertilization. It is another of those aspects of structure which emphasizes how little we know of the ultimate vital physiological activities associated with the processes revealed to us by the microtome. It would be interesting to clarify by careful technique the relation of the gynospore membrane and pollen tube wall to these projecting necks. Is the membrane here punctured? The nucleus of the male cell now rapidly increases in size (Pl. 13 : 7), the cytoplasm showing a very narrow clear zone just outside the nuclear membrane, a very distinct dense zone, a clearer peripheral zone, and a definite membrane. The dense zone—its chemical nature was not determined—has been somewhat overstained in the section from which the photograph was taken, but it is always very marked even in sections which have not yet been stained. In this section also the best plane for this male cell was just above a lower compact group of archegonia and just below an upper one, so that no archegonia appear in it.

Fertilization now takes place. Through a narrow channel between the neck cells a rush of cytoplasmic material from the male cell takes place into the archegonium, causing considerable disturbance in its upper cytoplasm, and displacing and somewhat distorting the female nucleus. The general features are shown in Pl. 13 : 8. The female nucleus is only just visible in this section, which is mainly to show the behaviour of the male cell, but would appear well in the next section of the series if reproduced. A varying quantity of cytoplasm leaves the male cell in different ovules. Here it can be seen that the denseness of the zone around the male nucleus has been greatly thinned, but it is still visible. The nucleus has not yet left the male cell. It will follow in separately shortly after. From the material available it seems reasonable to consider that the amount of male cytoplasm entering the archegonia is determined by the intensity of the first rush, the nucleus subsequently passing in more slowly with no further cytoplasmic addition, but this cannot be stated absolutely. The behaviour of the male cell here is very similar to that described by Lawson for *S. sempervirens*. The remains of the male cells outside the fertilized arche-

gonium, sometimes with little, sometimes with much, cytoplasm are clearly visible for quite some time. Three such appear in the one section in Pl. 13: 12. These cannot be confused with non-functioning male cells, as these latter either show obvious stages in degeneracy or show for a long time the typical male cell structure. The archegonia fertilized are usually side by side, and belonging to the more typically complex-like area of the group, but as the complex itself may be irregular, so the fertilized archegonia may be separated by regular prothallial cells. If more than one tube be present a corresponding number of fertilizations may be effected, but three pollen tubes with six fertilised archegonia were the greatest number actually seen in any one ovule.

*Proembryo formation.*

There is one point of structure in *Sequoia* which added a little to the difficulty of securing suitable preparations, applying to all stages, but particularly troublesome when endeavouring to follow the post-fertilization stages. If the form of the gametophyte is recalled it is clear that we have a group of archegonia radiating from a fairly spherical surface—the invagination. Obviously either in vertical or transverse or even oblique section only a few of the archegonia will be cut medianly in their whole length; most will be cut through some oblique plane. The archegonia also, never strictly symmetrical, may show degrees of bending and distortion with advancing development. Some stages, indeed, were never secured complete in median section in a form really suitable for photographic demonstration, and in too many cases the examples secured showing full median section of the archegonia at the stage desired were far from being the best examples of their kind. The difficulty is inherent in the structure of the material, and cannot readily be overcome, but it only affects the value of the photographic record, and not at all the statement of the actual conditions.

Proembryo formation is the stage of greatest interest in the development of *S. gigantea,* being definitely of a cupressinean type, and so differing markedly from that described for *S. sempervirens.* The male and female nuclei, approximately equal in size, pass to the lower part of the fertilized archegonium, and become surrounded by a dense cytoplasmic sheath. They are shown in contact in this position in Pl. 13: 9. No indication was seen of the separate division of the two nuclei, but frequently a large single nucleus formed from their fusion was met with, the large fusion nucleus being closely invested with the characteristic sheath. The first division takes place thus towards the bottom of the archegonium, and, as shown by the rather poor example in Pl. 13: 10, by the time this division is well advanced the denseness of the cytoplasm in the lower part has spread, so that now the archegonium is clearly divided into a lower third of firmer protoplasm to which proembryo formation will be limited, and an upper part of thinner vacuolated protoplasm occupying the upper two-thirds. No case was clearly seen at this stage of a persistent ventral nucleus. Two further divisions without wall-formation follow, giving thus

eight free nuclei in the lower part of the archegonium. This difference in proembryo formation from that described for *S. sempervirens* is so remarkable that two of the stages are reproduced photographically. Thus Pl. 13: 11, 12, show the binucleate stage. In the second of these very good examples are seen of the unused part of the male cell left in the pollen tube, there being three such present, in two of which a considerable quantity of protoplasm is still clearly distinguishable. The absence of a wall between the two proembryonic nuclei is evident. The succeeding figure, Pl. 13: 13, shows in a slightly oblique section the third division being initiated, again demonstrating the absence of walls. Several eight-nucleate stages were secured, but as never more than about five nuclei were naturally visible in one section, none are here reproduced. A rather complicated multi-polar spindle can be seen between the eight nuclei which return to an open resting stage without the appearance of any wall membrane on any of the spindles. It is doubtful if wall formation is at all associated with the spindle stage, but rather with subsequent cleavage planes. The eight nuclei are so arranged that, although not scattered irregularly, a certain readjustment of position takes place, resulting normally in a grouping of two or three below and five or six above, and it is quite clear in many cases that the spindles have completely vanished from between the upper nuclei before wall formation supervenes. There would appear to be here also the need for detailed cytological observation on the formation of the new walls, their relation to the existing archegonial wall, and, subsequently, the phenomena of wall elongation in those cells which form suspensors. Little accurate work is available here for any conifer. Normally there results a young proembryo with a closed tier of two or three cells below and an open tier of five or six cells above (Fig. 2 *a*). These stages are difficult to secure in median section, that shown in Pl. 13: 14, from a very poor preparation, being the most complete one available in a single section. It is a 2 : 6 arrangement. A transverse section through the open, walled, upper tier of a 3 : 5 proembryo is shown in Pl. 13: 15. As already mentioned, on the completion of the third division some readjustment of the location of the nuclei is necessary to give the normal type. Such readjustment may not take place properly, and irregular arrangements of eight-nucleate and eight-celled and intermediate stages may occur. As is usual, later proembryo stages are more frequent than earlier ones, but of twenty seen at this stage five showed a 3 : 5 type, seven showed a 2 : 6 type, and eight showed some marked degree of departure. Four such are seen in Fig. 2 *b, c, d, e*. A percentage of abnormal young proembryos is probably more widespread in the conifers than can be judged from published accounts. Normally in the 2 : 6 and 3 : 5 types the nuclei of the upper open tier divide again, giving a 2 : 6 : 6 or 3 : 5 : 5 arrangement—the rosette tier remaining open to the protoplasm which still occupies the upper part of the archegonium. Fig. 3 *a* represents this stage, and shows how similar the proembryo is to those recorded for cupressinean conifers with an apical complex. One of the sections from which it was drawn is shown in Pl. 13: 16. The three cells forming the

lowest tier may be practically in one plane, or may show a tetrahedral tendency, but there does not appear to be any regularity in the slight displacement of one of them; even when composed of two cells only they may not be exactly in one plane. It is of interest, though possibly merely accidental to these particular collections, that the 3 : 5 : 5 type was more than three times as numerous as the 2 : 6 : 6 type. Apparently also a number of the irregular eight-celled or eight-nucleate types may proceed to further division. About one-third of the

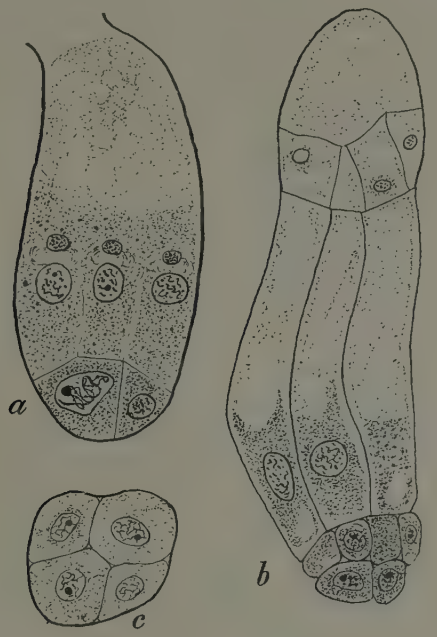


Fig. 3.—*Sequoia gigantea*. *a*. Normal proembryo in section at the 3 : 5 : 5 stage showing formation of the upper rosette tier by simultaneous division of the upper tier of the 8-celled stage. *b*. Normal proembryo at the early stage of suspensor elongation; there were 6 suspensors. *c*. Section of a neatly walled rosette tier composed in this case of only 4 rosette cells. All × 330. Aug. 24.

proembryos found between the eight-celled stage and the stage of suspensor elongation showed some abnormality. A few are shown in Fig. 4 *a, b, c, d, e*, Some of these are readily derived from an eight-nucleate stage, in others very irregular divisions have taken place. But few of these proceed further. Out of about forty-five proembryos seen with elongation of the suspensor tier only seven or so showed any abnormality, and of these some showed only a little irregularity and the others were doubtfully developing further, showing appearances of degeneration. In spite, therefore, of the presence of these abnormalities it is clear that, probably without exception, it is only proembryos

properly and normally tiered that proceed to further normal development. The type of that normal development is shown in Fig. 3 *b*. Even before the suspensor tier begins to elongate, further divisions may take place in the cells of the embryo tier. No regularity could be followed in them, but from four to seven cells were quite common. The suspensors may be 4, 5, or 6 in number—suppression of one or more of the cells of this tier being readily apparent from some of the sections; and 5 was again the commonest number. The nuclei of the rosette tier behave rather irregularly. In some cases one or two may degenerate, or they may divide, but eventually walls are formed

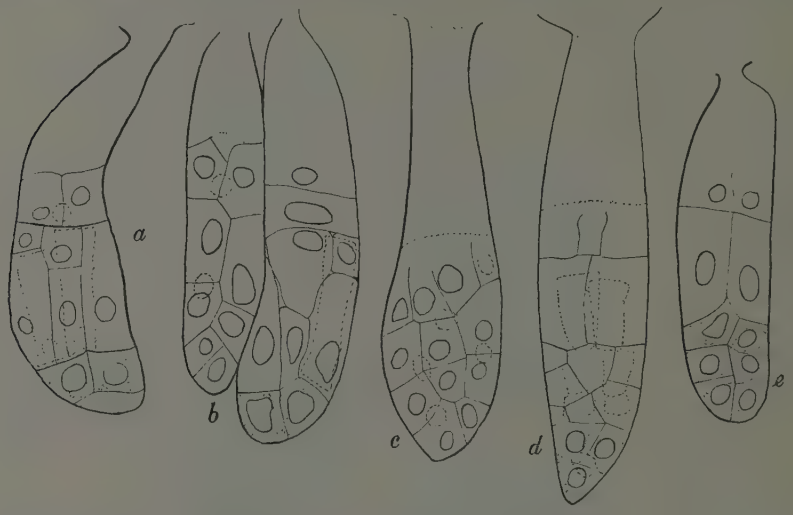


Fig. 4.—*Sequoia gigantea*. *a*, *b*, *c*, *d*. Examples of older abnormal proembryos, the embryo cells proper only shaded in when obviously distinct. *e*. Median section of an intermediate type with a clump of 7 or 8 embryo cells, 4 or 5 suspensors, some irregular, and perhaps 3 rosette cells. All × 240. Aug. 24.

building up a group of cells of no very definite arrangement. Nothing can be said as to the origin of these walls in relation to the nuclear division, if any. A section of a neatly walled rosette tier of only four cells is drawn in Fig. 3 *c*.

Consideration of the further development will be postponed. Some type of polyembryony ensues, which is at present being examined.

## B. *Sequoia sempervirens*.

The unexpected result of the study of the proembryo of *S. gigantea* refocussed attention on *S. sempervirens*. The statements of Arnoldi and Lawson on the early proembryo are not quite in agreement. According to Arnoldi, after fertilization the fusion nucleus passes to the bottom of the archegonium and there divides, while Lawson's Plate-fig. 33 shows division taking place well

up in the archegonium, in fact a little above the middle. This is the figure showing wall formation on the first division spindle; he does not, however, figure a two-celled stage. Lawson's next drawing, Fig. 34, shows a row of four cells, and is followed by Fig. 35, showing five cells, the description of the origin of the fifth being far from lucid. He noted the later occurrence of single suspensors, but did not explain the connection of this with his Fig. 35. Arnoldi, on the other hand, states that after the first division two cells are formed in connection with the two nuclei, the upper part of the protoplasm of the archegonium remaining without a nucleus, a point to which Lawson makes no reference. In fact, since the publication of Lawson's paper it seems to be generally taken that the whole archegonium of *S. sempervirens* is used in the formation of the proembryo. Arnoldi then further states that the lower of the two cells, which he describes as being organized in the lower part of the archegonium, divides, giving thus a row of three cells. The upper of these soon degenerates—the lower forms the embryo cell, and the middle one a single suspensor. The proembryo thus at certain stages appears two-celled, a condition drawn by him in his Plate-fig. 19. He thus accounts for the single suspensor.

These two accounts are obviously very different, and it seemed advisable to determine in so interesting a type as *S. sempervirens* which, if either, is the correct one, especially as Lawson's description, actually the less convincing of the two, is apparently the one commonly accepted.

It may be said, straightaway, that this species presented greater difficulties than *S. gigantea*. It grows vegetatively reasonably well, but shows, at least in the specimens available, considerable sterility in the reproductive structures at all stages. This may be associated with its polyploid nature, but it is just as likely to be due to the climate here not being suitable for it. The pollen actually tends to ripen under late winter conditions, the cold and wet resulting usually in most of it never properly maturing, and in that which matures being badly spread. Ovules at all stages show strong degeneracy tendencies, but this is particularly so at and after fertilization. Even if the ovules are still healthy, no definite date can be fixed for definite stages, some ovules being perhaps a fortnight later than others. Again, in healthy ovules two developments obscure the issue at the later proembryo stage. In this species jacket-like cells though scattered are fairly frequent, and these may become enlarged with dense protoplasm simulating proembryo cells. It is possible that Lawson may have confused some such cells with true proembryo ones, but without examination of his original slides such can only be suggested. In addition, not only jacket-like cells but neighbouring cells of the prothallus may become elongated and vacuolate, with a nucleus surrounded by dense protoplasm at one end, and frequently hardly to be distinguished from the true suspensors, which are usually single, stubby when young, and not very definite. The results of the examination of proembryo development here are, therefore, not as satisfactory as could be wished, but certain of the more definite points ascertained seem worth recording. Specimens with accessible cones in any number

are not common in the neighbourhood of Dublin. The material here examined was obtained from a tree in the grounds of Kilmacurragh, Co. Wicklow. As nothing was known of the dates at which critical stages occurred a great deal of material had to be worked through, and many collections made, involving for the results, such as they are, journeys totalling about 1,700 miles.

The mature gametophytes have been already described in general by Arnoldi and Lawson. Neither of these authors, however, describes properly the shape of the pollen tube, which differs but slightly from that described above for *S. gigantea.* The upper part of the tube is rather more slender, while the lower part is much wider. The unilateral swelling is larger and much less sharply marked off from the downward extension, which is shorter and wider. The body cells of the two species are very similar, and the male cells here show also clearly the denser zone of cytoplasm, although perhaps just a little less defined than in *S. gigantea.* This is shown in Pl. 14 : 17, which also indicates that the actual fertilization phenomena in the two species are similar, and not quite as described for *S. sempervirens* by Lawson, who gives the impression that the male nucleus is the active agent entering the archegonium accompanied by a little cytoplasm of the male cell. The point is a small one, and its real interest is to emphasize the very close similarity between the male gametophytes and the process of fertilization in the two forms. Lawson describes the pollen tubes as sometimes becoming embedded in the female prothallus. In the material here examined the upper part of the tube was always free, and definitely outside the gynospore membrane; the invagination was, of course, embedded to a certain extent, but complete enclosure of the tube by prothallial cells was only seen for the tail-like continuation of the tube below. It is not quite clear from Lawson's account how much of the tube may be embedded, and Arnoldi definitely states also that the tube is situated between the nucellus and the spore membranes. He makes no reference to any embedding of the tube in the prothallial tissue, and any of his figures showing either transverse or longitudinal sections at this stage are exactly paralleled from the material here examined. The point has this interest. In *S. gigantea* the tube is definitely outside the gynospore membrane and prothallial tissue. Both these records and those of Arnoldi make the same claim for *S. sempervirens,* emphasizing thus again the similarity of the gametophytes of these two species, and their difference from the callitrinean type. In this type not only is there no marked functional expansion of the tube, but the tube, according to the records of Saxton (7, 8, 9, 10), penetrates the prothallus even before cellular formation begins, and is, from an early stage, definitely embedded in that tissue. These two distinctly different groups of conifers, the *Callitrineae* and the *Sequoia* group, would appear, therefore, to have a gametophytic arrangement similar in general plan but markedly different in detail, this difference supporting that otherwise arrived at on systematic grounds.

As the female gametophyte develops it naturally does so to some extent at the expense of the nucellus which Shaw and Lawson describe as being reduced to its outer epidermis only, while Arnoldi says it is always composed of 5–7

layers of cells. Certainly any of the photographs here reproduced agree with the description of Shaw and Lawson. At some places occasional layers of inner cells are left, but the reduction is very complete, actually in many places the swelling gametophyte bursting through the nucellar epidermis. As already mentioned a firm many-layered nucellus is retained in *S. gigantea,* and this difference of structure has always permitted an exact separation of sections of ovules of the two species.

In regard to the female gametophyte its most obvious difference from that of *S. gigantea* lies in the gynospore membrane, which in *S. sempervirens* is quite delicate and usually thinner than the pollen tube wall. The whole gametophyte tends to be a little shorter and broader than that of *S. gigantea*; the archegonia show greater variation in size, and frequently tend to a short broad type seldom seen in *S. gigantea*; jacket-like cells, although irregularly scattered, are occasionally quite definite in appearance or in distribution round single archegonia or archegonial groups; the archegonia also tend to occur slightly more frequently unconnected with a pollen tube, and the indefinite complexes in association with the tube invagination tend to spread out from contact with the tube. These points can be seen in Pl. 14: 18, 19. Arnoldi describes considerable variation in the number of archegonia, these sometimes occurring in packed masses round the functional part of the tube. This has not been seen in the material here worked through, the archegonial number being approximately that of *S. gigantea* or a little greater. One ovule showed numerous archegonia clustered in the upper part of the prothallus right up to the apex. Arnoldi also records a similar appearance occasionally occurring. In this case the whole prothallus was strikingly similar to one described elsewhere in a form as widely separated from *Sequoia* as *Fitzroya* (4), the only important difference being the position of the pollen tube. These points of structure have been of course more fully described by Lawson and Arnoldi, the latter's account being the better of the two, and only referred to here to emphasize, as in the case of the male gametophyte, the close similarity between the female gametophytes of the two species, with the exception of the gynospore membrane.

Shortly after fertilization, the male and female nuclei can be seen in contact at the bottom of the archegonium. Three photographs are reproduced of this stage, as it is one of the points in which Lawson's account differs from Arnoldi's. In the first two, Pl. 14: 18, 19, the appearance is quite cupressinean and agrees with the statement of Arnoldi. Comparison with Pl. 13: 11 shows how similar this stage is in *S. sempervirens* to that in *S. gigantea.* The third photograph shows a point of further interest. The two nuclei can be seen in contact surrounded by a dense zone of cytoplasm; towards the neck of the archegonium the cytoplasm appears as composed of two zones thinner than the basal zone, and distinctly different from each other, the zone nearest to the neck being a little more compact. Reference to Arnoldi's figure of the first division after this stage shows an exactly similar layering of the cytoplasm. Although the first division was not met with, yet from the appearance of the

archegonia at the stage here described in comparison with the appearance when the first division is completed, it is quite certain that Arnoldi's statement that the fusion nucleus divides towards the base of the archegonium is quite correct. Actually, the male and female nuclei control a certain quantity of the cytoplasm in the lower part of the archegonium. The cytoplasm left in the upper part of the archegonium varies considerably in amount and also in duration, sometimes breaking down and completely vanishing shortly after the first division, sometimes definite and distinct to the later proembryo stages. The records of Arnoldi and Lawson that the first division results in the formation of two cells are of course quite correct, as was only to be expected from the definiteness of their statements. This stage is shown in Pl. 14: 21. This section was definitely not good, showing a great deal of contraction, but it was the only fully median one secured among a number cut obliquely; the same difficulty, only in a more pronounced form, of securing complete median sections for photography occurring here as in *S. gigantea.* As Lawson does not figure this critical two-celled stage, and as Arnoldi's drawing is rather diagrammatic, this figure is here reproduced in order to put on photographic record until a better one is secured the two cells arising in connection with the first division, and to show the unused degenerating cytoplasm left in the archegonium. It thus illustrates and supports the statement of Arnoldi concerning the immediate results following the first division—"rings um zwei hier liegende Kerne herum enstehen zwei Zellen während der obere Teil des Eiprotoplasmas ohne Kerne bleibt." In this case also a definite small quantity of cytoplasm at the bottom can be seen. It is separated by a membrane, which however does not show in the photograph, from the two cells. Unfortunately, owing partly to the technique employed, but mainly to the poverty of the material available, detailed cytological observations on the formation of these cells could not be made, but it seems probable that these two cells are cut out from the archegonial cytoplasm somewhat in the manner described for *Ephedra* (5). A second division results in four cells. The two divisions seem usually to take place in one of three ways. The upper nucleus may divide in the long axis of the archegonium and the lower transversely, giving rise to the appearance shown in Pl. 14: 22. In this case the non-nucleate protoplasm has already disappeared. In the second type the upper nucleus divides transversely, the lower nucleus in the long axis. An example of this is shown in Pl. 14: 23, supplemented by the drawing Fig. 5 *a*. This example, although rather a poor one, is reproduced as it shows the divisions in progress, the upper non-nucleate protoplasmic area and another such area cut off at the side below. A third type is shown in Pl. 14: 24 resulting from an oblique division of the two nuclei. In this case a considerable area of unused protoplasm is left in the upper part of the archegonium. Actually this protoplasm was much more obvious in the next section of the series, this one being chosen as showing the four-celled proembryo much more clearly. The condition of four cells in a row as described by Lawson was never seen. Arnoldi had described the proembryo as composed of three cells in a row, the non-nucleated protoplasm having by this stage apparently disappeared, although

such is not definitely stated. It is most probable that Arnoldi was really dealing with sections through proembryos such as those here figured. Slightly oblique sections through any of these would readily give a row of three cells, so that again Arnoldi appears to have observed more accurately than Lawson. It is clear, so far, that the proembryo is normally four-celled. The type shown in Pl. 14: 24 was less common than the other two, and, it should be remembered, even where this type occurs, the amount of unused protoplasm at this stage is usually less. This figure, however, seems to suggest that, in general, apart from the stage of wall formation, the various cupressinean proembryos differ in development merely in the varying amount of the lower cytoplasm of the archegonium controlled by them. All leave a volume of unused cytoplasm in the upper part of the archegonium, cytoplasm which may vary considerably in amount and in viability, and which may show the extreme condition of permanency and extent, in some cases at least, if a ventral canal nucleus remains in an active condition. Re-examination of material of *Athrotaxis* and *Fitzroya,* in which a completely septate archegonium has been reported (4, 11), has shown (data not yet published) that these records are in error. It is possible that a similar error has been made in the recording of fully septate archegonia in the callitrinean forms. As it is also hoped to discuss elsewhere

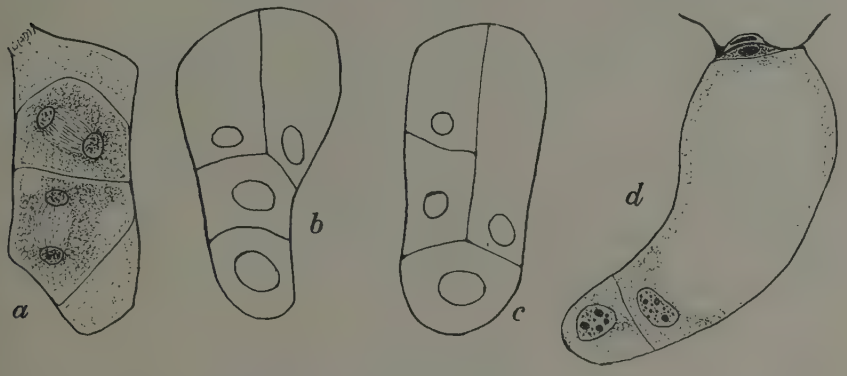


Fig. 5.—*Sequoia sempervirens. a.* Second division in proembryo giving one of the types of 4-celled stage described in text. × 240. Aug. 11. *b, c.* The two abnormal later proembryos, diagrammatic. × 240. Aug. 13. *d.* Typical older proembryo. × 330. Aug. 10.

this and other points of structure as they bear on our ideas of the phylogenetic relationships within the wide group of conifers with cupressinean affinities, this aspect will not be further discussed here; it seems desirable, however, to call definite attention to it. In all the proembryos of *S. sempervirens* the unused protoplasm degenerates, whether slowly or rapidly (sometimes very rapidly), and unless a fair series of young stages is available can be easily overlooked, thus readily giving the impression that a four-celled proembryo is formed. using completely the archegonial cytoplasm, such a type for example as is figured in Pl. 14: 22.

Arnoldi further states that the upper of the three cells described by him degenerates early, the second elongating to form the suspensor, the third forming a single embryo cell. Again Arnoldi's statement can be definitely supported. In practically all the later proembryos here seen, allowing for the four-celled instead of the three-celled arrangement, the upper one or two cells according to the proembryo type seem rapidly to break down. There may thus be found later proembryos showing one suspensor with one or two embryo cells, or showing two suspensors with one embryo cell. The commonest type is drawn in Fig. 5 *d*, and one of the sections from which this figure was drawn is photographed in Pl. 14: 25. As Arnoldi says, referring to his own Plate-fig. 19, the proembryo at this stage appears to be composed of two cells only. His Plate-fig. 19 and our Fig. 5 *d* and Pl. 14: 25 might readily have been reproduced from the same section. The condition with two suspensors and one embryo cell was rare, but that may have been due merely to the poverty of the material. Owing to the small amount of material available, and the marked sterility which seems to set in about this stage, it was not possible to feel satisfied about the later developments. Examples showing one suspensor with one, two, or a clump of embryo cells, as described also by Arnoldi, were common, but it cannot yet be definitely stated if any form of cleavage polyembryony occurs. There may, however, as in *S. gigantea*, be variants from the typical proembryo. Only two examples have so far been seen, both outlined diagrammatically in Fig. 5, *b, c*. The first would seem to be a variant on the type shown in Fig. 5 *a*, with the upper cells functioning as suspensors, the second a variant on that shown in Pl. 14: 24 with three upper cells elongating, one much more actively than the other two, which have come to lie end to end. If plentiful material were available it might be possible to decide whether such variants are more common than they appear to be here, and, if many occur, whether any of them develop to maturity. There was strong evidence in *S. gigantea* that they do not. But only further collections of more satisfactory material can settle such points as these or properly elucidate the later normal embryology.

## Conclusions.

The two species *S. gigantea* and *S. sempervirens* closely resemble each other in the structure of the mature gametophytes, male and female, as well as in the mechanism of fertilization. They differ in other respects. *S. sempervirens* completes its development in one year, *S. gigantea* taking two. The gynospore membrane of the latter is very distinct, in the former quite fine. But the really important difference lies in the proembryo formation in the two species, as here described. This raises a point of some interest. There is not yet among students of the conifers an agreement, even provisionally, as to what can be taken as criteria of structure for the purpose of phylogenetic comparison. Can the proembryo be used as such a criterion, and within what limits? Proper discussion of any suggested criteria needs comprehensive treatment for which

this is not the place; it is only intended here to indicate the nature of the problems raised. The regularly tiered proembryo of the *Abietineae* seems a fairly definite characteristic in the case of that group, but there is no such uniformity within the cupressinean conifers, using the general adjective cupressinean to cover the whole complex commonly included in the *Taxodineae* and the *Cupressineae*. The genera *Sequoia* and *Athrotaxis* are undoubtedly, both in structure and development, very closely allied, but a proembryo with wall formation at the binucleate stage is found in *S. sempervirens,* one with wall formation at the four-nucleate stage in the three species of *Athrotaxis,* and one, of a very cupressinean type, with wall formation at the eight-nucleate stage in *S. gigantea.* If major emphasis be laid on the proembryo this completely natural group must be broken up into three, *S. sempervirens* being put separate, *S. gigantea* perhaps transferred to close relationship with *Taxodium* and *Cryptomeria,* while *Athrotaxis* would need transference to the *Callitrineae* or to a close relationship with *Fitzroya.* Such a separation is impossible, and if the proembryo be valueless in estimating the relationship of *Athrotaxis* to *Sequoia,* and still more of the two species of *Sequoia* to one another, it would seem that, if used at all in a consideration of the affinities within the cupressinean complex, it can only be used as a criterion of very subsidiary importance. It is, therefore, difficult to suggest, on the basis of this proembryo difference alone, that *S. gigantea* should be more fully separated from *S. sempervirens* under the distinct generic name of *Wellingtonia* by which it is so often called. This point, however, can be considered later, when the details of the prothallial development in the two species have been re-examined. On the other hand *Sequoia* and *Athrotaxis* are very similar in their gametophytes. The *Callitrineae* also, grouped on the basis of cones and habit, have this grouping supported by their gametophyte form, a point to which reference has already been made. The typical cupressinean prothallus, characterized by an apical complex, runs uniformly through the *Cupressineae* proper, a group always linked together systematically. In the *Abietineae,* also, the gametophytes show a nice uniformity throughout the group. The gametophytes, therefore, would appear to be of much greater value in comparing relationships. The case of *Microcachrys,* however, shows that the gametophytic structure cannot always be accepted without question as an important criterion. This form, always included in the *Podocarpineae,* differs entirely from *Podocarpus, Dacrydium,* and *Saxegothaea,* in its gametophytes, which are of a strict cupressinean type. Are we therefore to doubt its podocarpean nature? The records of development in *Sequoia* would seem, therefore, to open the way for a full examination of the criteria of comparison in the *Coniferales,* an examination long overdue.

## Summary.

1. The mature gametophytes of *Sequoia gigantea* are described. They resemble in general those of *S. sempervirens,* the gynospore membrane, however, being much thicker.

2. Fertilization is similar in the two species. A rapid inward flow between the archegonial neck cells carries a varying quantity of cytoplasm into the egg. This is followed later by the inward passage of the male nucleus, leaving much of the protoplasmic contents of the male cell outside.

3. The fused male and female nuclei, in *Sequoia gigantea,* lie and divide in the bottom of the archegonium giving rise to eight nuclei, at which stage walls are formed to give two tiers 2 : 6 or 3 : 5. The upper tier divides again. The proembryo is thus cupressinean in type.

4. Revision of proembryo formation in *S. sempervirens* shows that Arnoldi's account is more accurate than that of Lawson. The fusion nuclei lie at the bottom of the archegonium, the first division undoubtedly giving two cells, but the protoplasm of the upper part of the archegonium is not used, and gradually degenerates. A second division gives a four-celled proembryo showing in a basal direction a 2 : 1 : 1, or 1 : 1 : 2, or 1 : 2 : 1, arrangement. The upper cell or cells break down, the next cell or cells give a single or double suspensor, the basal a single or double embryo cell.

5. Some of the questions raised by this proembryo difference, such as the relative importance of proembryo and gametophytes as criteria of phylogenetic comparison in the conifers, are indicated.

Thanks are due to Lord Powerscourt for permission to collect in his estate; to the owners of Kilmacurragh for a like permission; and to the firm of Mason and Sons, Dublin, for the preparation, from negatives supplied to them, of the prints used for the photographic reproductions in the plates.

## EXPLANATION OF PLATES.

(All × 125 except Figs. 1, 2, 18, 19 which are × 55 and 8, 10, 13, 21 which are × 250.)

### Plate 13.

*Sequoia gigantea.*

(All Aug. 24 except 2, 4, 5 which are Aug. 21.)

1.—L.S. of part of ovule to show the invaginating bulge of the pollen tube, the downward extension and the general position of the tube lateral to the female gametophyte.

2.—T.S. of ovule a little below the invagination, and excluding the integument except for its inner epidermis, to show the many-layered nucellar tissue and the distinct gynospore membrane with three pollen tubes outside it.

3.—T.L.S. of small part of female gametophyte to show the four neck cells of the archegonia.

4.—T.S. of fertile area of the gametophyte to show the body cell, cut in its long axis, and two archegonia in contact forming a partial complex, the archegonia having projecting necks.
5.—Body cell sectioned in its short axis.
6.—T.S. of fertile area to show the young male cell and the projecting neck of an archegonium.
7.—Mature male cell in section shortly before fertilization.
8.—Archegonium and male cell in section to show first phase of male cell entry at fertilization. The archegonial nucleus, just seen here, is quite distinct in the next section of the series.
9.—Male and female nuclei in contact at the base of the archegonium after fertilization.
10.—Archegonium in section to show first post-fertilization division.
11, 12.—Sections of proembryos to show the binucleate stage and, in 12, three male cell remnants left in the pollen tube.
13.—Slightly oblique section to show the third division in a fertilized archegonium and the absence of walls at this stage.
14.—Section of an 8-celled proembryo *in situ* with a 2 : 6 arrangement.
15.—T.S. through the upper tier of five cells of a 3 : 5 proembryo.
16.—Section from a slightly oblique series of a 3 : 5 : 5 proembryo to show formation of the rosette tier.

## PLATE 14.

### *SEQUOIA SEMPERVIRENS.*

17.—Male cell in section just initiating fertilization, the associated archegonium appearing in another section of the series. Aug. 11.
18, 19.—T.S. of complete gametophytes enclosed in nucellus showing the general arrangement of the parts described in the text, and particularly the male and female nuclei, after fertilization, in contact at the base of the archegonium. Aug. 7.
20.—Another archegonium in section showing the same stage. Aug. 7.
21.—Two-celled proembryo in median section, showing also the residual archegonial cytoplasm. Aug. 7.
22, 23, 24.—T.S. through the archegonial area of three different ovules to show in median section the three types of proembryo described in the text. Aug. 12.
25.—Later proembryo in section to show the single embryo cell, the single swollen suspensor, and the two upper cells of a 4-celled stage degenerated and seen as a small black area which is really double. Aug. 12.

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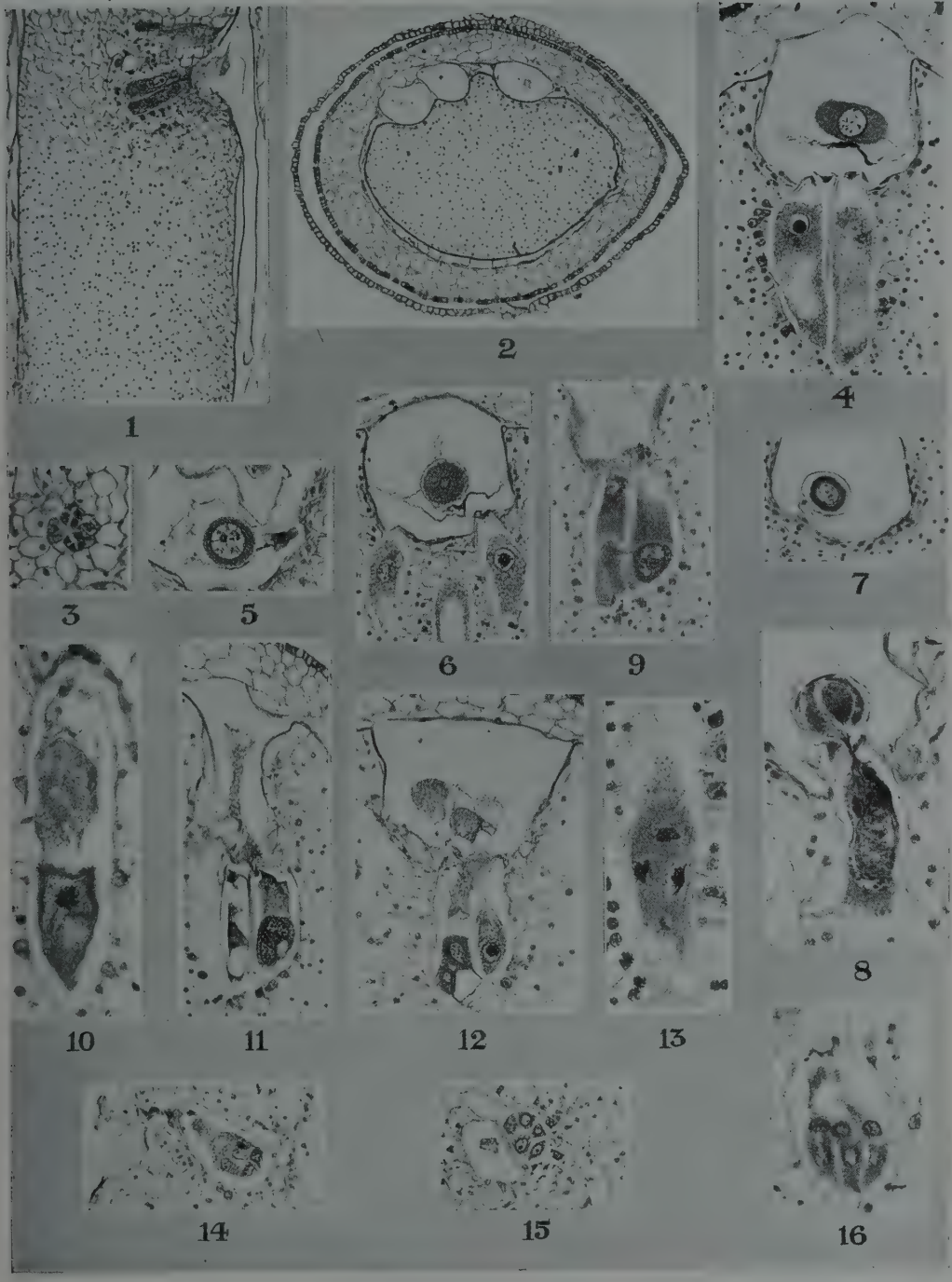
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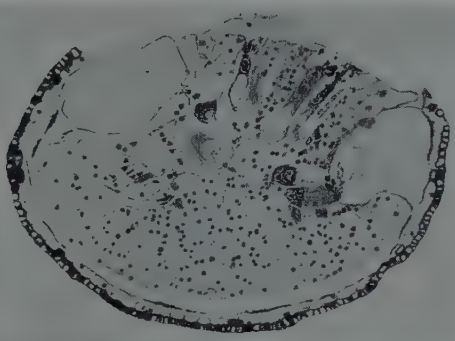

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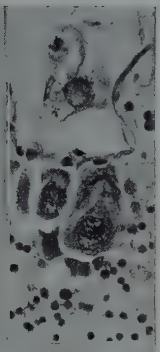

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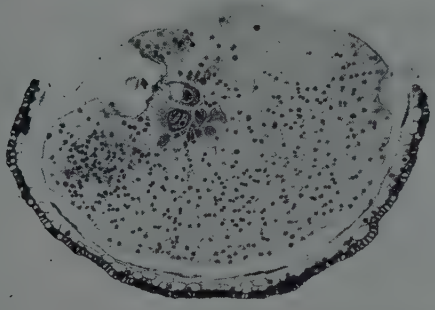

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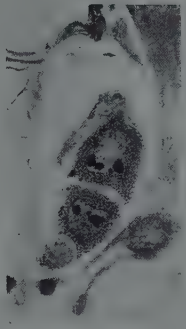

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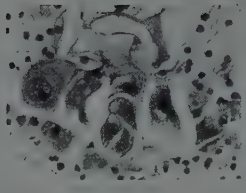

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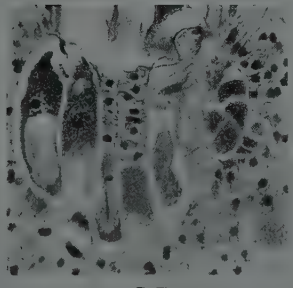

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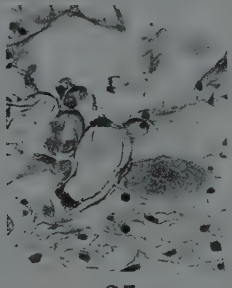

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No. 45.

# THE CONVECTION OF HEAT AND MATERIALS IN THE STEM OF A TREE.

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[Read November 24. Published separately December 29, 1936.]

The thermoelectric method of measuring the velocity of flow in tubes was, according to Huber, first used by H. Rein (3 and 4) for determining the rate of flow in blood-vessels. In 1932 Huber (2) himself used it for investigating the velocity of the transpiration current. About the same time it occurred to me that thermocouples might be applied to the detection of the flow of material in the bark of trees, if such flow occurred. Many things conspired in preventing me from testing this idea. However, towards the end of the summer of 1935 I was able to make a beginning, and I commenced more or less regular observations in November of that year.

The method essentially consists in the local application of heat to the presumed channel of the moving liquid, and the detection of this heat by means of a thermocouple at a point on the channel at a measured distance from the source of heat. As Huber (2) and Baumgartner (1) used it, a considerable distance intervened between the heater and the point of detection. This was arranged so that the junction might not be affected by the heat *conducted* by the intervening tissues or by the hot air rising from the heater, which was below the couple.

This disposition of the heater and couple limits the method to the observation of comparatively rapid velocities. For, during the slow passage of the fluid through a considerable length of intervening tissues, the heat will become gradually dissipated, and will not be revealed by the couple towards which it is travelling. In Huber's and Baumgartner's experiments on the transpiration current, distances of 4 cm. to 25 cm. intervened between the source and the couple, but as they were dealing with velocities of 5 cm. to 30 cm. per minute this was no disadvantage. Where velocities of 1 or 2 cm. per min., or even less, are suspected it may be necessary to cut down the distance considerably, so that, while the heated liquid moves from the source to the couple, all of its added heat will not be lost. In reducing the distance there is a danger that the heat *conducted* through the tissues may lead to a rise in the

temperature of the nearer junction of the couple, and this may be confused with the looked for rise due to the *convection* of heat by the moving liquid.

Prolonged observation of the behaviour of the thermocouple itself fortunately may be utilized to eliminate within limits the danger of this confusion. Heat cannot be *conducted* from a colder to a hotter region. So that if the junction more distant from the source of heat becomes hotter than that which is nearer, it is certain that heat has come to it, not only by *conduction*, but also by *convection* through the tissues.

Hence, when a momentary application of heat locally to a stem leads to a temperature-rise in the more distant (above that of the nearer) junction, it indicates a movement of material upwards or downwards in the stem, according as the recording thermocouple is placed above or below the heater. Further, the velocity of the movement may be estimated by noting the interval between the moment of heat-application and that when the temperature of the distant junction exceeds that of the nearer one.

Relying on this principle, I have been able to detect movement as slow as 2 cm. per hour with thermojunctions 1 cm. apart, and with the near junction only 1 cm. from the heater.

The heater in these experiments consisted of a piece of German silver wire (0·2 mm. diam.) about 10 cm. long. Insulated with celluloid it was folded on itself 8 times and bound together tightly with a fine silk thread. Its ends were soldered to the copper leads coming from a transformer supplying a current

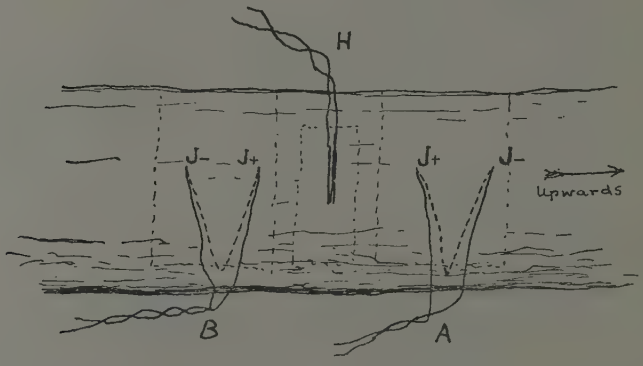


Fig. 1.—Diagram showing the arrangement of the heater (H) and of the upper (A) and lower (B) thermocouples. J + and J – the junctions of the thermocouples. The dotted lines indicate the outlines of the pieces of adhesive plaster fixing the heater and couples in place. The arrow shows the direction of the apex of the branch.

at 2 volts. This heater was brought over the nearly horizontal experimental branch, and fixed in close contact to the *intact* bark on one of the vertical sides of the branch by means of a strip of adhesive plaster. By this disposition the

air heated by the German silver tends to rise clear of the thermocouples which lead in from below. These latter are fixed, without any disturbance to the plant's tissues, by similar strips on the same horizontal line of the branch as the heater, and on each side of it. The arrangements are made clear by reference to Fig. 1. When the heater and the couples are fixed, the portion of the branch to which they are attached is thickly wrapped round with cotton wool. To protect the couples further from external heat fluctuations and from rain, the whole is enclosed in a piece of waterproofed canvas. The upper end of this is bound down on a collar of plasticine moulded against the branch and turned back overlapping the canvas.

The thermocouples which I used were made of constantan and copper; the terminals of the copper leads to the galvanometer form the copper elements, thus reducing as far as possible the number of junctions in the circuit, which are each apt to introduce error. The terminals of the leads in the laboratory form connection with a key, or switch, by which the different couples may be put in circuit with the galvanometer. In order that the unavoidable junctions in the switch may not introduce thermoelectric error, the terminals are enclosed in a thick-walled copper box, so that these junctions will be kept at the same temperature.

The galvanometer available for the work was a moving coil, A. & M., galvanometer, having a resistance of 20·8 ohms. Its sensitivity is 340 mm. per microamp. at 1 metre. With the thermocouples used and the scale 50 cm. from the mirror, a temperature difference between the junctions of one degree centigrade gave a deflection of 150 scale-divisions.

Changes in the zero with this arrangement proved a great trouble. These changes might amount to as much as 18 scale divisions in six hours. Presuming that they were chiefly due to the recovery from strains in the suspension of the galvanometer, it was first sought to eliminate them by frequent readings of the position of rest of the mirror when the circuit was broken, and, by correcting the readings of the experimental couples by the observed alterations of this zero.

Subsequent experiments showed that other disturbances—viz., those associated with the circuit—contributed to this error. These did not appear when the circuit was broken, and so were not allowed for when the circuit was open. Hence, to eliminate both those due to the suspension and to the circuit, a third couple was installed in close proximity to the two experimental ones under the protection of the cotton-wool and canvas. The junctions of this third couple, insulated by a film of celluloid, were brought into close contact with one another and were wrapped around with a good thermal conductor (copper or lead foil). With its junctions thus maintained at the same temperature this couple gave a zero modified only by the suspension and the circuit. The leads of the three couples were then formed into a single cable, running from the branch to the switch in the laboratory.

With these arrangements a zero, corrected for the strains in the galvanometer suspension and for accidental potentials picked up by the leads, was

obtained. Fig. 2 shows graphs in which the readings of the open circuit zero (O.Z.), of the closed circuit (C.Z.) couple, and of the two experimental couples (A and B), are compared at various times. During these observations the two junctions of each of the three couples were kept at the same temperature, being in close proximity, and enclosed in a good conducting sheath. In the graphs the vertical scale recording the galvanometer deflections is ten times that in the other figures of this paper, and it is evident that, while the open

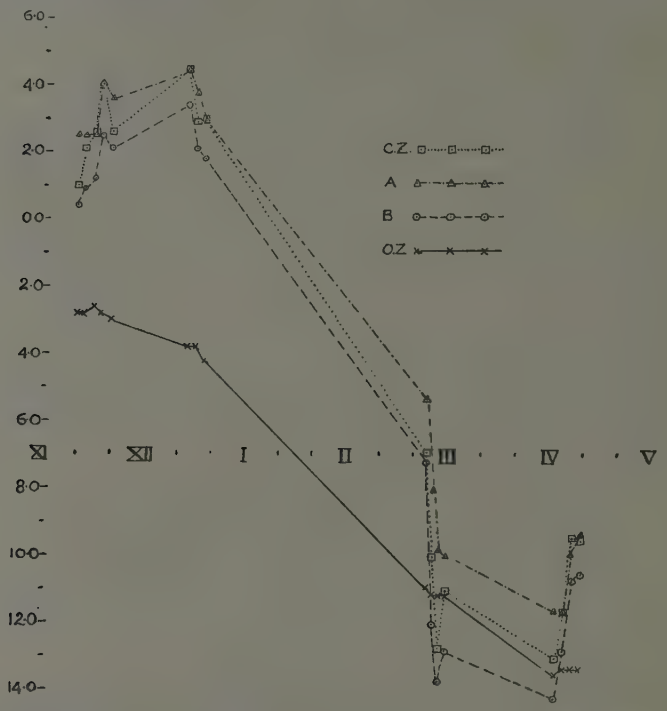


Fig. 2.—Graphs showing the variations of the zeros of the three thermocouples A, B and C.Z. and of the zero of the galvanometer with open circuit during six hours. The junctions of each thermocouple were maintained at the same temperature.

circuit zero varies in the same sense as the zero of the thermocouples, the correction afforded by the C.Z. couple in fine details is much more satisfactory.

The experimental tree is a young ash (*Fraxinus excelsior*), about 7 metres in height, the stem being about 15 cm. in diameter at the base. The branch serving for the observations is about 2 metres from the ground, and has a diameter of 5 cm. The tree stands about 10·5 m. to the N.E. of the laboratory in a sheltered angle between two walls. It is exposed to the sun during the forenoon and early afternoon. Later the shadow of a building more or less cuts the tree off from direct sunshine. A wooden screen was arranged to shade

the almost horizontal part of the branch under observation during the earlier part of the day from direct sunshine; and the couples and heater were fixed on the north aspect of this branch. The observations recorded began in November, 1935, and were continued with unavoidable interruptions till October of this year.

The thermocouples affixed to the branch were so arranged that, when the junction nearer to the heater was at a higher temperature than the more distant one, the current passing in the circuits caused a positive deflection of the galvanometer. Thus, when the switch of the heater current was closed momentarily (viz., for 5, 10, or 30 secs.), at first a positive deflection was observed. This grew in magnitude till a maximum was attained. Then, as the heat reached the more distant junction, the deflection declined towards zero; and, if the more distant junction became the hotter, the deflection became negative, and usually then tended to return to zero. Thus, when the deflections are plotted in a graph against time, two different types of curve are traced. In the first (Type **I**) the positive deflection declines toward the zero line, but does not reach the starting level (Fig. 3, Feb. 19, curves A and B). In the second, it not

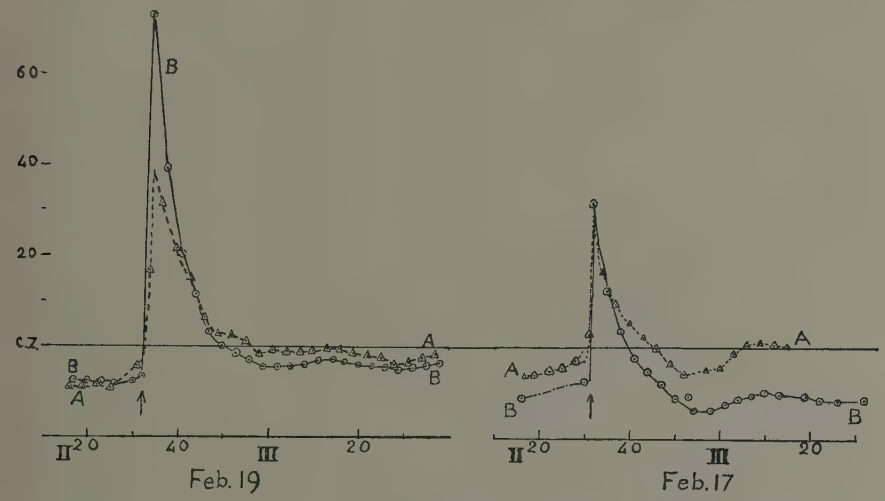


FIG. 3.—On left: curves traced simultaneously by A, upper and B, lower thermocouples on Feb. 19; neither shows convection of heat; Type I a. On right: curves traced simultaneously by A (upper) and B (lower) thermocouples on Feb. 17; both show convection of heat; Type II a. The arrows indicate the moment of the application of heat.

only reaches this level, but actually passes it (Type **II**). As was pointed out already, the latter form of curve indicates that heat has been *conveyed* to the more distant junction, granted that external disturbances are eliminated. Fig. 3, Feb. 19, curves A and B illustrates the first type of curve; while Fig. 3,

Feb. 17, curves A and B, and all the curves on Fig. 4, exhibit examples of the second type. When Type **II** is encountered we may be sure that there has been convection of heat, and hence movement of material in the stem. If the curve traced is the result of observations with a couple fixed above the heater, then that movement has been upwards (see graphs marked A); if it has been defined by observations made with a lower couple, the movement has been downwards (graphs marked B).

Variations in these two types occur. First, with regard to Type **II**: in the early parts of the year the dip below the starting level, on the declining arm of the curve, is gradual, as is also its climbing back again to the original level. This form may be designated as type **II** a. It is exemplified in Fig. 3,

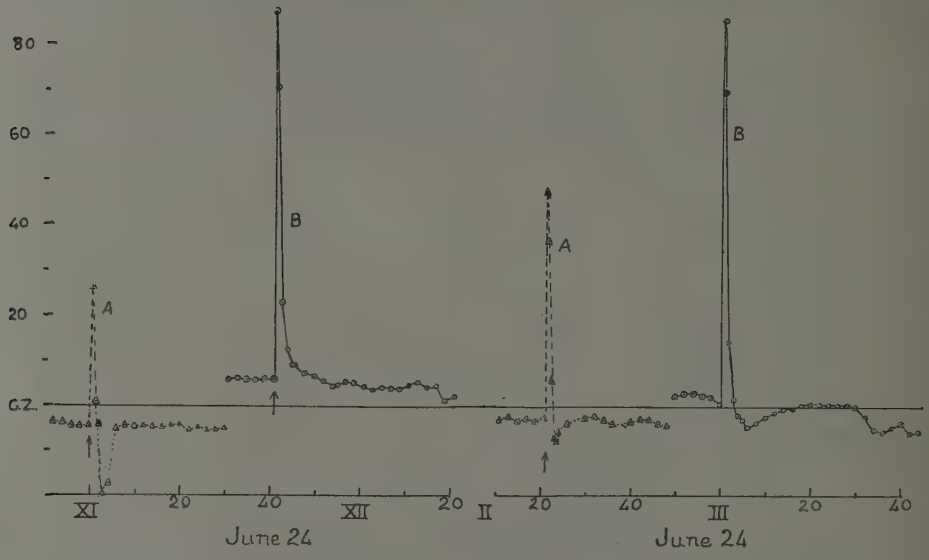


FIG. 4.—On left: Curve A shows upward and B shows downward convection. The steep minus deflection in Curve A indicates the rapid convection in the transpiration stream with a velocity of approximately 2 cm. per minute. Curve B shows a slower velocity downwards, viz., 1 cm. per 5 minutes. On the right: Curve A shows upward movement similar to that shown by Curve A on the left. Curve B shows a velocity downwards of 80 cms. per hour.

Feb. 17, graphs A and B, and Fig. 5, May 27, night A, and is the usual form of curve traced by the lower thermocouple, and by the upper one except on summer days, when transpiration may be assumed to be rapid. It probably is the result of slow movement of heated material gradually coming to the temperature of the surrounding tissues. Type **II** b, exemplified by graphs A in Fig. 4, shows a very rapid recovery from the first deflection, and an almost equally rapid return from the negative excursion. It appears to be associated with a rapid movement of heated material, which traverses the distance between

the heater and the distant junction so quickly that little spread of heat has occurred in the moving substance or into the adjacent tissues. Type **II** b is regularly found on summer days in the tracings of the upper couple. Less

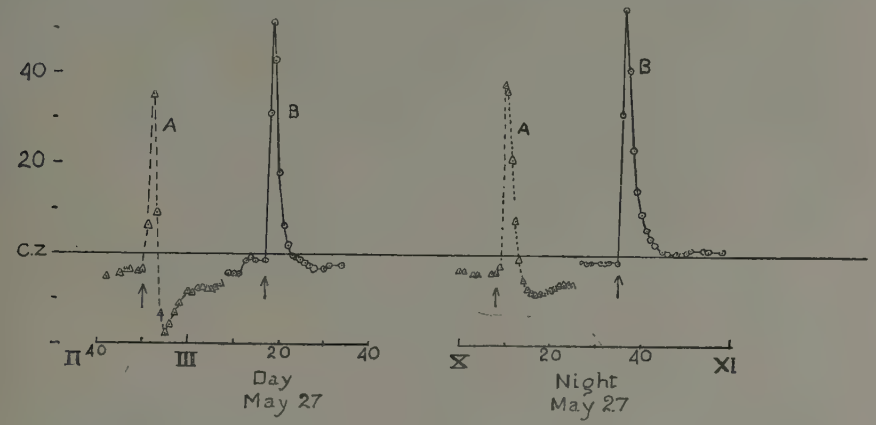


FIG. 5.—Curves traced by upper (A) and lower (B) thermocouples during the day on the left, and during the night on the right, May 27.

emphasised examples are also produced by the lower couple, especially during the summer months (Fig. 4, graph B on the right). Sometimes, even when the curve on its return from the maximum positive deflection does not attain the original level, it may show an inflection or a group of inflections where it begins to assume an asymptotic direction. Such curves may be assigned to Type **I** b (see Fig. 6, May 15, graph A). It seems probable that, in some cases,

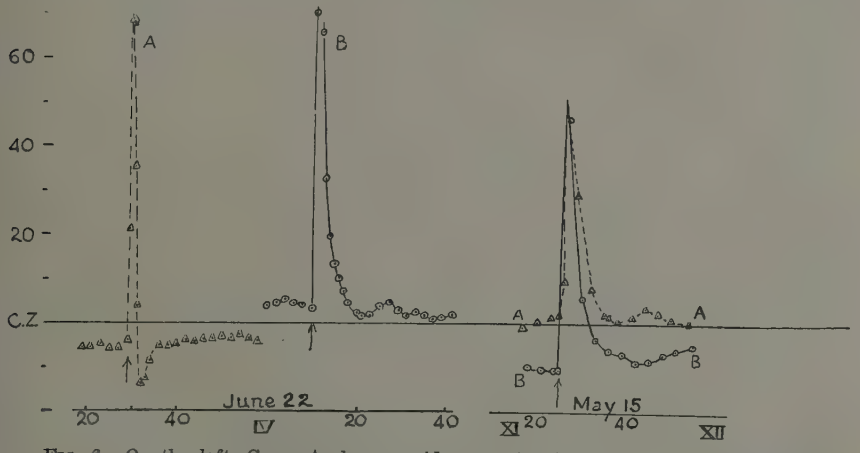


FIG. 6.—On the left, Curve A shows rapid convection in transpiration current and curve B slower movement downwards. On the right, curves A and B show inflections as they approach their starting levels which possibly indicate slow convection.

these inflections indicate the arrival of a certain amount of heat conveyed by a moving column, which, however, is not sufficient, owing to dissipation, to bring the curve back to the starting level. Such experiments are not entered in the table as showing upward or downward movement. Other curves almost reach the starting level, but do not pass it, running along practically at the starting level (Fig. 5, night B); they are assigned to Type **I** a, and, of course, are not assumed to show convection.

TABLE.

| Month. | No. of Tests Made. | No. of Tests Showing Upward Motion. | Average Upward Velocity. | No. of Tests Showing Downward Motion. | Average Downward Velocity. |
|---|---|---|---|---|---|
| Nov., 1935 | 10 | 7 | 8·5 cm. per hour. | — | — |
| „ „ | 37 | | | 21 | 6·1 cm. per hour. |
| Dec., „ | 10 | 7 | 11·5 „ „ | | |
| Jan., 1936 | 2 | 1 | 2 1 „ „ | | |
| „ „ | 2 | | | 2 | 2·7 „ „ |
| Feb., „ | 21 | 11 | 6·7 „ „ | | |
| „ „ | 21 | | | 13 | 4·9 „ „ |
| March, „ | 13 | 7 | 4·4 „ „ | | |
| „ „ | 13 | | | 6 | 6·7 „ „ |
| April, „ | 8 | 6 | 5·9 „ „ | | |
| „ „ | 8 | | | 4 | 5·9 „ „ |
| May, „ | 33 | 28 | 19·3 „ „ | | |
| „ „ | 33 | | | 21 | 8·6 „ „ |
| June, „ | 14 | 14 | 37 5 „ „ | | |
| „ „ | 14 | | | 11 | 13·3 „ „ |
| July, „ | 4 | 4 | 38·0 „ „ | | |
| „ „ | 4 | | | 3 | 28·6 „ „ |
| Aug., „ | | | | | |
| Sept., „ | 2 | | | 2 | 13·3 „ „ |
| Oct., „ | 8 | 4 | 11·8 „ „ | | |
| „ „ | 3 | | | 1 | 22·2 „ „ |
| Year | 125 | 71% | | | |
| „ | 137 | | | 61·4% | |

49·5 per cent. of the experiments arranged to record simultaneously upward and downward movements gave positive results in both directions.

The table gives a record of the experiments made, showing those which indicated upward and downward convection and the velocities of the convection on different dates. Where the two couples, one above the heater and one below, were supplying records, the two results are entered. At the earlier dates only one was installed, and so only upward *or* downward movement could be detected.

The table also shows the proportion of positive results obtained in each month. During the year 262 tests were recorded; 125 of these could show upward and 137 downward movement, if such occurred. Positive evidence was afforded in 71 per cent. of the experiments which were arranged to detect upward movement, and, of those arranged to detect downward movement, positive results were given in 61·4 per cent. Of 95 experiments arranged to detect upward and downward motion 49·5 per cent. gave positive results for simultaneous movement in both directions.

The results quoted seem to establish the fact that there is mass movement in the stem both upwards and downwards. At the start of the work marked movement was expected only in the summer months, when transpiration is taking place. What is surprising is the evidence that mass movement takes place both upwards and downwards even while the tree is bare of leaves and during the winter months. Except for the upward transpiration current in summer months, the movements appear to be spasmodic.

The question as to the tissues which provide the channels for the moving matter naturally obtrudes itself. The very rapid movement upwards shown in the experiments made during May, June, and July immediately indicates that the transpiration current in the wood provides a vehicle for the heat upwards. This view has been confirmed by a few experiments, where extirpation of the bast above the heater did not markedly diminish the downward sweep of the recording curve below the starting level. Hence, with suitable reservations, we may at present assume that that part of the curve, during the months of May, June, and July and later, records the convection of heat in the transpiration current. This is interesting, as showing that the thermoelectric method can record the approximate velocities of the transpiration current in perfectly intact plants which have been subjected to no organic interference. The few experiments where the bark below the heater was extirpated did not contradict the view that the downward movement observed was in the bark. In them the curves given were of Type I a. Unfortunately, time has not yet been available to repeat these experiments sufficiently often to get by this means conclusive evidence on this point, and as the records show, several experiments might be made without finding downward movement, even although there had been no interference with the bark. If, however, we concede the probability that the convection of heat downwards takes place in the bark, we will naturally assume that the convection upwards also is in the bark during the leafless months, when transpiration is in abeyance. This view is supported by the similarity of the curves recording the upward and downward movement when the leaves are off the tree, and by the similarity at night of the upward to the downward curve (see Fig. 5).

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No. 46.

# STUDIES ON THE ULTIMATE COMPOSITION OF BIOLOGICAL MATERIAL.

## PART I: AIMS, SCOPE, AND METHODS.

By DAVID ALLARDICE WEBB and WILLIAM ROBERT FEARON.

(Plate 15.)

[Read March 23. Published May 18, 1937.]

An inquiry into the distribution of the chemical elements within the kingdoms of life and an examination of their possible significance as constant or variable constituents are of both general and special interest. It is a contribution to one of the broader aspects of geochemistry, the study of the laws governing the dispersion of the elements in space and the extent of their diffusion or concentration, which may vary greatly among the different species of organism, quite apart from the scarcity or the abundance of the element.

The chemical importance of organisms as geological agents has long been recognised by Clarke and his colleagues in America (Clarke, 1920, Clarke and Wheeler, 1922), and by Vernadsky (1930, 1935), Vinogradov (1935), and their associates at the Biogeochemical Laboratory of the Academy of Sciences of U.S.S.R., since 1910; while the study of what may be termed "biochemical morphology" is of fundamental importance in determining the manifestation of organic life.

It is only within the last twenty-five years that serious attempts have been made to explore systematically the ultimate composition of the organism by micro-chemical and spectrographic methods; and, although a large number of analyses have been published, it is not yet possible to compile with certainty a complete list of the elements present in animals and plants. This difficulty is due partly to the scattered and inaccessible nature of much of the relevant literature, especially that of Vinogradov, most of which awaits translation from the original Russian; partly to the contradictory results obtained by different workers, and to the undoubted errors in some of their methods; partly to the inadequacy of the results when considered in relation to the vast field to be surveyed; and, most of all, to the scarcity of even approximate quantitative

data. Presumably, if analytical technique could be sufficiently refined, the presence of every element could be detected in every organism, if only to the extent of a few thousand atoms; and the statement that a given element is "present" in one species and "absent" from another may give unwarranted prominence to comparatively small differences in concentration which happen to include in their range the limits of sensitivity of the particular analytical method employed. Thus, if it were demonstrated that a marine animal contained $10^{-12}$ per cent. of radium, this would only indicate that the animal had no specialised mechanism for excluding radium from its body, since $10^{-12}$ per cent. is the approximate radium content of sea water, its natural environment.

For an element to be accepted as significant, quite apart from any physiological or pathological effect it may exert, it must either be shown to participate in the life of the organism or else must be present consistently in concentrations much greater than those found in the normal environment.

## The Biological Elements.

It is possible, if no claims for finality be made, and if a few very exceptional organisms be ignored,[1] to compile a table of the biological elements and to classify them in accordance with two criteria: their constancy and variability among the species, and their quantitative distribution. This has already been attempted by one of us (Fearon, 1933) in an earlier paper, and the same tabulation has been followed in the present revision. It must be realised, however, that for some of the elements the number of different species analysed by reliable methods is insufficient to enable one to decide whether an element is strictly variable or invariable, and, furthermore, that the quantitative classification, though convenient, is necessarily arbitrary.

Quantitative assessment is often rendered uncertain by the difficulty in deciding how far exo- and endo-skeletons and similar stable tissues can be reckoned as part of the actively living organism. For example, the quantity of silicon in the body of a diatom may considerably exceed the quantity of carbon, but though the absorption of silica is of great importance in the metabolism of such organisms, that does not displace carbon from its primary position in all organic structure.

This classification it will be seen accepts eighteen invariable elements and twenty-one variable elements, making a minimal total of thirty-nine biological elements altogether. Vinogradov (1935) has raised the total to sixty, but his additions are mostly what he terms "ultra-micro" constituents, and will not be discussed in the present paper.

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[1] Many of these aberrant forms are listed in the chemical classification of organisms given by Vernadsky (1923).

TABLE I.

Biological elements classified according to their distribution as percentage body-weight of the organism.

| INVARIABLE (18) | | | VARIABLE (21) | | |
|---|---|---|---|---|---|
| Primary ($1 - 60\%$) | Secondary ($0{\cdot}05 - 1\%$) | Micro-constituents ($< 0{\cdot}05\%$) | Secondary | Micro-constituents | Contaminants (?) |
| Hydrogen | Sodium | Boron | Titanium | Lithium | Helium |
| Carbon | Magnesium | Fluorine | Vanadium | Beryllium | Argon |
| Nitrogen | Sulphur | Silicon | Zinc | Aluminium | Selenium |
| Oxygen | Chlorine | Manganese | Bromine | Chromium | Gold |
| Phosphorus | Potassium | Copper | 4 | Cobalt | Mercury |
| 5 | Calcium | Iodine | | Nickel | Bismuth |
| | Iron | 6 | | Germanium | Thallium |
| | 7 | | | Arsenic | 7 |
| | | | | Rubidium | |
| | | | | Strontium | |
| | | | | Molybdenum | |
| | | | | Silver | |
| | | | | Cadmium | |
| | | | | Tin | |
| | | | | Caesium | |
| | | | | Barium | |
| | | | | Lead | |
| | | | | 17 | |

When the above classification was first proposed, in 1933, attention was drawn to the fact that most of the biological elements are of low atomic weight, and that a line, continuous though devious, could be drawn across the periodic table separating it into two groups: an upper group of biological and a lower group of non-biological elements. A revised form of this table is now presented, in which two lines are drawn, dividing it into three regions: *invariable biological elements,* above the broken line; *variable elements,* between the two lines; and *non-biological elements,* below the continuous line.

Two elements, lithium and beryllium, do not fit properly into this scheme, since, in spite of their position above the broken line, neither can be regarded as generally essential for life. Indeed, it is remarkable that, although the biological elements as a class are distinguished by their low atomic weight, three of the four lightest elements should be scanty in distribution. Lithium occurs in most marine organisms and in a few terrestrial animals, but usually in minute concentrations, little, if at all, greater than those found in the environment. With beryllium, the anomaly resides in the plan of the periodic tabulation; for beryllium, although a member of the calcium group, in many ways resembles the metals of the aluminium group, which are of remarkably little use to the organism. Vinogradov reports (1932) the presence of beryllium in

certain plants, and one of us (Fearon, 1933) has detected it in the ash of two species of lichen; but on account of the great difficulties involved in the complete separation of plant material from associated rock dust it would be unsafe to regard this evidence as conclusive.

TABLE 2.

Biological elements classified according to position in the Periodic Table.

| Group 0 | Group I a | Group I b | Group II a | Group II b | Group III a | Group III b | Group IV a | Group IV b | Group V a | Group V b | Group VI a | Group VI b | Group VII a | Group VII b | Group VIII a | Group VIII b |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | H 1 | | | | | | | | | | | | | | | |
| He 2 | Li 3 | | Be 4 | | B 5 | | C 6 | | N 7 | | O 8 | | F 9 | | | |
| Ne 10 | Na 11 | | Mg 12 | | Al 13 | | Si 14 | | P 15 | | S 16 | | Cl 17 | | | |
| A 18 | K 19 | | Ca 20 | | Sc 21 | | Ti 22 | | V 23 | | Cr 24 | | Mn 25 | | Fe 26 Co 27 Ni 28 | |
| | | Cu 29 | | Zn 30 | | Ga 31 | | Ge 32 | | As 33 | | Se 34 | | Br 35 | | |
| Kr 36 | Rb 37 | | Sr 38 | | Y 39 | | Zr 40 | | Cb 41 | | Mo 42 | | Ma 43 | | Ru 44 Rh 45 Pd 46 | |
| | | Ag 47 | | Cd 48 | | In 49 | | Sn 50 | | Sb 51 | | Te 52 | | I 53 | | |
| Xe 54 | Cs 55 | | Ba 56 | | La* 57 | | Hf 72 | | Ta 73 | | W 74 | | Re 75 | | Os 76 Ir 77 Pt 78 | |
| | | Au 79 | | Hg 80 | | Tl 81 | | Pb 82 | | Bi 83 | | | | | | |
| Rn 86 | | | Ra 88 | | Pa 89 | | Th 90 | | | | U 92 | | | | | |

If these reserves be made, the elements can be classified physiologically as follows:—

(1) *Indispensable elements,* which are necessary for life in any form.
(2) *Contingent elements,* which are available as soon as a suitable biological mechanism has been evolved to deal with them.
(3) *Replacement elements,* which can supplement or substitute the contingent elements.
(4) *Unavailable* and *non-biological elements.*

It will be seen that with one exception the heavy metals, well-known for their toxicity, belong to the last group. Lead, the exception, according to Spiro (1925), owes its inclusion among biological elements to the readiness with which it forms harmless compounds by union with lipoids. This is supported by the biological location of lead, chiefly in organs such as brain, which have a high lipoid content, and by the fact that, although the metal is highly toxic

to purified enzyme preparations, crude preparations of urease are able to withstand high concentrations of lead salts (Fearon, 1936).

The organism's power of neutralising or immobilising harmful metals probably operates in many ways and with many different elements. Löhner (1924) has shown that organisms with a high content of copper held in organic combination are just as susceptible to the toxic effects of copper ions as are organisms that are not copper-storers.

It is sometimes asserted that in the absence of any clear evidence of precise physiological function the survey of the micro-constituent elements of the organism is little more than an analytical exercise. But the existence of the hormones and the vitamins is a sufficient proof that what is quantitatively minute is not necessarily insignificant.

Quite apart, however, from any problems of function, it is probable that the study of the elemental composition of the organism and the environment will prove to be a fruitful method of investigating the general principles which determine the way in which the organism regulates its composition. How and where are the biological elements absorbed, stored, and excreted? What factors control their distribution in the body-fluids? Does chemical relationship involve biological relationship? What associations exist between the micro-constituents and the various catalyst-systems of the organism? The purpose of this our present paper is to propound these and other questions, and to show the methods most likely to provide the answers.

## Methods of Analysis.

For the detection and estimation of the minor constituents of living tissues microchemical methods, usually colorimetric or nephelometric, have been and are being widely applied. Within the last ten years or so, spectroscopic methods of attack have been studied with increasing success, as demonstrated by Ramage and his colleagues (Fox and Ramage, 1931; Sheldon and Ramage, 1931; Ramage, Sheldon, and Sheldon, 1933), and by various Continental and American workers. The great advantages of the spectrographic methods are as follows:—

1. All or most of the elements can be searched for simultaneously. This saves time, and is also especially important when only a small sample of material is available. A representative analysis, for example, has been made, using the lens of a single eye (Burdon-Cooper and Lewis, 1929).
2. Certainty of identification is very great. Chemical tests are always liable to be confused or inhibited by the presence of associated reactants, but in spectroscopy, provided a line is correctly identified, no such ambiguity is possible.
3. Unforeseen elements can be detected and estimated as easily as those for which a search is being made.

4. Although for many elements the limits of detection are not so small as those reached by refined micro-chemical methods, the absolute quantity that can be detected is very minute, often of the order of $10^{-7}$ g., and sometimes less. And if suitable methods of concentration are employed previous to spectrographic examination the range can be extended much further.

Against these great advantages must be set the two principal defects of the spectroscopic method:—

1. Certain elements escape detection owing to the fact that their atoms cannot be excited to emit radiation by the means usually employed. These elements are all non-metals, and from the biological aspect only two are of significance—namely, bromine and iodine.

   The remaining halogens can be dealt with by other methods. Fluorine, although it shows no lines in the arc spectrum, when combined with calcium readily gives the band characteristic of the $CaF_2$ molecule. Chlorine usually occurs in concentrations sufficient to respond to the ordinary chemical tests when they are applied directly to the tissue extracts; and the recent diffusion method, introduced by Conway (1935), has made it possible to estimate chlorine and the other halogens in very small amounts of material.

2. Reliable and accurate quantitative data are hard to obtain. The present-day methods of quantitative spectroscopy under most favourable conditions may attain an accuracy of 5 per cent. in the estimation of an element present in about 2 per cent. concentration (Way and Arthur, 1935). In most cases the probable error is considerably larger.

   In the present state of our knowledge, and especially when we consider the very wide degree of individual variation that may be displayed by the minor constituents of the lower animal organism (Phillips, 1917), this disadvantage is not as serious as may appear. It is unprofitable to strive after a one per cent. accuracy if the next sample of the material examined shows a content differing by twenty-five per cent. A knowledge of the order of magnitude in which the constituents occur is likely to be as useful as an absolute quantitative finding.

Common alike to chemical and spectroscopic methods of analysis is the danger of contamination from environmental, manipulative, and other sources, each type of which will be considered in turn.

### *Environmental Contamination.*

It is very difficult to separate many organisms completely from associated material. Since the interest of the analysis often lies in the difference or

resemblance between the composition of the organism and the composition of its environment, contamination from the latter is a serious source of error, the importance of which some investigators appear to ignore. Thus, it has been claimed (Azéma and Pied, 1930) that the blood of certain tunicates contains aluminium and silicon. Unless this blood has been collected directly from the blood-vessels, a matter of very great difficulty owing to the small size of the animals examined, it seems more reasonable to conclude that these elements are present in the form of clay particles which are found in the bodies of all feeders on plankton and detritus, both on external surfaces and within the gut.

We have found, for example, that the inorganic ash of the earthworm *Lumbricus* still shows evidence of soil contamination when spectrographed, even after the animals have been fed on ashless filter paper for over a fortnight. It is probable that very porous or arborescent organisms, such as sponges and lichens, cannot by any mechanical method be adequately freed from extraneous contaminants; so that, though specific elements may be searched for if they are scarce in or absent from the environment, a general analysis of the organism for secondary and micro-constituents is untrustworthy in its results.

To avoid errors from this source it is necessary, on the one hand, to select for analysis only such specimens as can be effectively freed from environmental material; and, on the other hand, to examine all positive findings critically, with the object of recognising contamination when it does occur. We have systematically rejected results showing an abnormally high content of both aluminium and silicon, for, although these are both undoubted biological elements, they never occur in large quantities (organisms with siliceous skeletons, of course, being excepted), and their simultaneous appearance in equivalent proportions may be ascribed with certainty to the presence of particles of rock or clay. For organisms living in sandy environments the content of silicon alone forms a criterion of the purity of the tissue examined. The method used by Fox and Ramage (1931) in their important pioneer work on biological spectroscopy of marine invertebrates did not allow of the detection of either aluminium or silicon; and, since many of the species and tissues examined (*Clathrina,* sedentary polychaetes, and gills of lamellibranchs, for example) are liable to contain large amounts of detritus which the cleaning methods adopted were insufficient to remove, it is probable that certain of their results were vitiated by environmental contamination.

### *Manipulative Contamination.*

Manipulative contamination may be due to the reagents employed as well as to the instruments used in preparing the tissues. One of the advantages of spectroscopy is that very little preliminary treatment of the analytical material is necessary: at most, preservation in alcohol, addition of a few drops of nitric acid during ashing, and addition of ammonium sulphate afterwards. These reagents may be obtained in forms sufficiently pure for spectrographic use, and their purity may be controlled by blank experiments throughout the series of analyses. The best practical guarantee of absence of contamination by reagents

is found in the fact that no suspect elements appear constantly throughout the analytical series. The dangers of fortuitous contamination from dust, apparatus, and personal manipulation, though they are sometimes exaggerated, must not be overlooked. Thus, we have found that if an exposure were made, using a silver arc, any subsequent exposures made in the same room and on the same day, using a carbon arc, were liable to show evidence of the presence of silver. Finally, there is in spectroscopy the continual risk of contamination from impurities present in the electrodes used to obtain the arc or the spark illumination. The chief advantage of the methods employed by Ramage (1929) and by Lundegårdh (1934) is the substitution of the gas or hydrogen flame for the electric arc. It is now possible, however, to obtain silver electrodes sufficiently pure to give a spectrum free from all foreign lines—apart from a very faint indication of the presence of calcium. Silver electrodes unfortunately have two disadvantages when compared with carbon or graphite. Owing to the lower temperature of the silver arc the sensitivity is less for most of the elements, and, of course, the method cannot be used for the detection of silver. We have found it advisable to employ both silver and carbon arcs concurrently. The interpretation of the spectrograms given by the carbon arc, however, requires critical care, since impurities are present always, and these may falsify the result in a way not previously realised owing to a "sensitising effect," described in the Appendix to this paper and elsewhere (Webb, 1937).

A detailed description of the technique employed will be given in the next paper of the series. For the present, it is sufficient to state that the instrument used was a Hilger medium quartz spectrograph (E 488), which, while giving a dispersion adequate to resolve almost all pairs of lines likely to cause confusion, enabled the entire spectrum from 2,000 A. to 10,000 A. to be photographed by one exposure. Using panchromatic plates, a working range of 2,250 A. to 7,000 A. was obtained.

The material was ashed in silica crucibles at as low a temperature as possible, no attempt being made to burn off all the carbon. In all other respects the technique adopted was that of Lewis (1932, 1933). The ash was then spectrographed in the silver arc, and, for many of the observations, in the carbon arc as well. Quantitative data, when required, were obtained by comparison of the spectra with those given by standards of known composition.

Elements that presented features of biological interest were checked by means of micro-chemical tests applied either to the ash or to the fresh material. The application and scope of these tests as a supplement to spectroscopic work will be made the subject of a later communication.

## The Organism and its Elemental Environment.

In order to delimit the frontiers of study and to map out the field of profitable investigation, certain type materials were first selected for examination. The results of these preliminary analyses are submitted in the present paper as possessing an intrinsic interest, and at the same time serving to illustrate some of the problems which may be attacked by spectrographic methods.

### *The Organism as a Geological Agent.*

During its brief and insecure habitation of the earth's surface, life has left many traces of its occupation. Some of these, such as fossils and coal-seams, are visible memorials; others are only revealed by chemical analysis. Thus, we may regard the presence of porphyrin derivatives in shales and asphalts (Treibs, 1934, 1935) as evidence that plants have played a major part in the formation of petroleum; and Vinogradov has collected considerable evidence of the organogenic accumulation of iodine, vanadium, and other biological elements in various parts of the territories of life. Among the organogenic deposits, peat is, perhaps, the most familiar in this country. It possesses a two-fold interest, for its composition is a record of the selective activity of organisms in the past, and is, at the same time, an environmental medium for organisms of the present day.

As far as we are aware no complete analysis has previously been made of the elements present in peat. The material selected was from a cutting in the bog of Allen; one sample being taken from just below the surface, one from a depth of five feet, and one from a depth of nearly twenty feet. We are indebted to Professor E. A. Werner for these samples in the form of ash. The analyses are presented in Table 3, and the spectrograms are reproduced in Plate 15, 1 to 5.

The variation in composition is very striking. There is a continuous decrease in sodium and the micro-constituents lead and tin, and an increase in the proportion of iron and the alkaline earth metals, the increase in iron being sufficient to impart a distinctive reddish colour to the ash from the lowest level. The most remarkable features are the apparent absence of potassium and the presence of silver. Potassium admittedly has a low arc sensitivity, but even when this is taken into account even the uppermost samples of ash must contain much less than one per cent. of the metal.

Peat consists largely of the remains of forest timber, which is not nearly as rich in potassium as are the soft, rapidly growing tissues of green plants. The detection of silver is in accordance with the results obtained by de Rubies and Lemmel (1935), who, in a spectrographic analysis of the wood of twenty-six species of trees, found silver present in seventeen species, including members of genera common in Ireland, such as *Alnus, Fraxinus, Fagus,* and *Pinus.*

TABLE 3.

Composition of Peat Ash at different levels.

| Element | Al | Ba | B | Ca | Cu | Fe | Pb | Li | Mg | Mn | Ni | Si | Ag | Na | Sr | Sn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Surface | ++ | (+) | + | ++ | + | ++ | + | (+) | +++ | ++ | – | ++ | + | ++ | + | + |
| 5 ft. | ++ | + | + | +++ | + | +++ | (+) | (+) | +++ | ++ | ? | ++ | + | + | + | – |
| 20 ft. | ++ | ++ | + | ++++ | (+) | ++++ | ? | (+) | +++ | ++ | – | ++ | (+) | (+) | ++ | – |

In these, and subsequent tabulations, (+) denotes a faint but detectable trace of the element.

Aquatic organisms are surrounded by an approximately constant medium, which, in the case of sea water at any rate, contains a very large number of elements in low concentrations. Terrestrial animals, on the other hand, have a less completely passive environment, and depend on their food intake for their entire mineral supply. Since the domestic herbivora form a metabolic clearing house between man and the grass of the field, it is instructive to survey the micro-constituents available in their diet. Table 4 shows that hay ash contains most of the elements recognised in animal tissues, and supports the popular belief that the food plants act as necessary accumulators of biological elements.

TABLE 4.

Composition of the ash of Meadow Hay.

| Element | Al | Ba | B | Ca | Cr | Cu | Fe | Pb | Mg | Mn | Mo | Ni | P | K | Si | Ag | Na | Sr | Sn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amount | (+) | (+) | ? | ++ | (+) | + | + | + | ++ | ++ | ? | (+) | + | +++ | +++ | (+) | ++ | (+) | ? |

*Biological Discrimination between Closely Related Elements.*

Living organisms often exhibit powers of exact discrimination between elements of the same group, and it has even been claimed by Russian workers that this discrimination extends to the isotopes of certain elements, notably potassium. From a medium much richer in magnesium than in calcium, certain marine species are able to secrete a skeleton of calcium carbonate containing only 0·1 per cent. of magnesium. Another example of discrimination is found in the seeds of *Bertholletia excelsa,* the Brazil nut, which was found by Seaber (1933) to contain a relatively high concentration of barium. According to Werner, the barium content of the testa is even greater than that of the endosperm, which was the only tissue examined by Seaber.

It was decided to supplement these chemical observations by a spectrographic survey of three regions of the nut: the main woody part of the testa, the inner membranous part of the testa which is adherent to the endosperm, and the endosperm itself. The results are presented in the following table:—

TABLE 5.

Percentage composition of seed ash of *Bertholletia excelsa.*

| Element | Ba | Al | B | Cu | Fe | Pb | Mg | Mn | Ni | P | K | Na | Zn | Si | Sr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Woody part of Testa | 3·0 | ++ | 0·05 | 0·04 | 0·08 | 0·01 | ++ | 0·08 | + | – | +++ | + | – | ++ | 0·75 |
| Inner part of Testa | 8·0 | – | 0·08 | 0·15 | 0·15 | – | ++ | 0·15 | ? | ? | +++ | + | – | – | 0·16 |
| Endosperm | 0·03 | – | 0·08 | 0·3 | 0·3 | – | +++ | 0·05 | + | +++ | ++++ | + | + | – | 0·1 |

The presence or absence of an element not directly estimated is denoted by the signs + and –. A double + and a triple + denote concentrations of the orders of 0·1 and 1·0 per cent. respectively, in the majority of instances.

These results show that the inner part of the testa is the region richest in barium, while the endosperm is the poorest, and actually contains more strontium than barium.

The constancy of the Ca : Sr ratio throughout the nut and the variability of the Ba : Sr ratio are seen from the following figures:—

Ca : Sr.—*outer testa* 80, *inner testa* 80, *endosperm* 80;
Ba : Sr.—*outer testa* 4, *inner testa* 50, *endosperm* 0·3,

showing that the plant is able to discriminate between barium and the other alkaline-earth metals, but not between calcium and strontium, since the value of the Ca : Sr ratio is of the same order as that usually found in soils. The analysis in its entirety serves as an illustration of the fact first pointed out by Ramage (1936), that different regions of a seed may vary widely in composition.

The restriction of aluminium and silicon to the outer part of the testa we ascribe to environmental contamination.

*Racial Discrimination by Closely Related Organisms.*

Vinogradov and his colleagues have collected extensive data in support of their claim that "the chemical elementary composition of the organism is a species character" (1935). It appeared to us to be of interest to examine the composition of two such closely related organisms as baker's and brewer's yeast, in which a fundamental metabolic difference resides, unaccompanied by any marked difference in cytological structure.

TABLE 6.

Composition of the ash of Baker's Yeast and Brewer's Yeast (approximate percentages).

| Element | Al | Ba | B | Ca | Cr | Co | Cu | Fe | Pb | Li |
|---|---|---|---|---|---|---|---|---|---|---|
| Baker's Yeast (Dublin) | 0·1 | 0·015 | – | +++ | – | 0·005 | + | ++ | 0·04 | (+) |
| Baker's Yeast (Various) | 0·1 | 0·015 | – | +++ | – | 0·005 | + | ++ | 0·0008 | (+) |
| Brewer's Yeast (Dublin) | 0·01 | 0·002 | 0·02 | ++ | 0·001 | – | (+) | ++ | – | – |

| Element | Mg | Mn | Mo | P | K | Si | Na | Sr | Sn | Zn |
|---|---|---|---|---|---|---|---|---|---|---|
| Baker's Yeast (Dublin) | +++ | 0·007 | + | +++ | +++ | ++ | +++ | (+) | 0·02 | 0·3 |
| Baker's Yeast (Various) | +++ | 0·007 | – | +++ | +++ | + | +++ | + | – | 0·3 |
| Brewer's Yeast (Dublin) | ++ | 0·001 | – | +++ | ++++ | + | ++ | (+) | – | – |

Four different specimens of baker's yeast were analysed: a sample as marketed in Dublin, and samples as supplied to the baking industry in Cork, Liverpool, and Holland. Of these, the last three gave results that were identical within the limits of error, while the first had a much higher lead content, and showed the presence of molybdenum and tin, neither of which could be detected in the other yeasts. These differences, of course, may be due to contamination during processing.

The brewer's yeast was of Dublin origin, and in its mineral composition differed markedly from the other yeasts, notably in its low content of aluminium, barium, cobalt, lead, manganese, copper, and zinc, and in the fact that it contained boron and chromium. The percentages given are those determined spectrographically, and only represent orders of magnitude.

## APPENDIX.

### On the Presence of "Latent" Impurities in Graphite Electrodes.

(By D. A. Webb.)

As has already been pointed out elsewhere (Webb, 1937), a phenomenon has come to light during the course of these researches which has an important bearing on the effect of certain impurities found in graphite electrodes. Though apparently unnoticed hitherto, this is of considerable significance in the interpretation of spectrograms obtained with such electrodes. It consists simply in the observation that the procedure, recommended in most of the practical text-books of photographing, by means of a Hartmann diaphragm, a blank spectrum of the electrodes alone in juxtaposition with the spectrum of the specimen is not an adequate safeguard against impurities in the electrodes being taken to be actual constituents of the specimen.

The reason for this is that the presence in the arc of a saline ash is liable to render the lines of certain of the impurities in the electrodes far more prominent than they are in the spectrum of the electrodes alone.

The graphite used was Hilger's "H.S." brand (lab. no. 9968), and was accompanied by full spectrographic and chemical reports on its composition. The spectrographic report stated that when an arc was struck between the electrodes (under conditions very similar to those employed in our analyses) the following elements showed one or more lines in the spectrum:—B, Ca, Fe, Mg, Si, Na, V, Li, and Cu. A number of other elements could be detected chemically or spectrographically in the graphite ash, but were not present in sufficient quantities to appear in the spectra of the graphite arc. Actually, it was found that some of the elements mentioned in the spectrographic report showed up so feebly that they could only be detected in favourable and fairly long exposures. This applied specially to vanadium and copper. It was assumed that if stronger or more numerous lines of these elements could be seen in the spectrum of a specimen, then it was safe to infer their presence in the specimen.

Suspicions were first aroused by the fact that all the tissues examined appeared to contain titanium and vanadium; but as both have been often reported in biological material it was thought that the explanation might be that the spectroscopic method was unusually sensitive with regard to these elements, and their distribution was wider than had been supposed. Next, however, the spectrograms of the synthetic standards were examined, and it was seen that the lines of these two elements showed little or no tendency to become fainter as the proportion of the element in the mixture was progressively reduced. Finally, a comparison was made between the spectrograms obtained by

(1) Exposure to the graphite arc alone.

(2) Exposure for an equal length of time to the graphite arc into which had been introduced some spectroscopically pure sodium chloride (Hilger's Specpure), whose purity had been tested by examining its spectrum in the silver arc.

The result, shown in Plate 15, revealed the very different behaviour of the impurities in the electrodes in the two cases. The introduction of the sodium chloride modifies profoundly the spectrum of the electrodes by greatly intensifying the principal lines of iron, copper, calcium, vanadium, and (though not visible on this plate, since a panchromatic plate was not used) lithium, while several lines of titanium, which are completely absent from the "blank" spectrum, are rendered plainly visible on the negative, though they are too faint to be seen in the reproduction. On the other hand, the lines of magnesium, silicon, and boron are unaffected by the introduction of the sodium chloride, or sometimes slightly reduced in intensity.

The interpretation of this phenomenon in terms of ionisation and resonance potentials will not be attempted, but it is clear that the introduction of a large number of easily ionised atoms, such as those of sodium, is bound to modify profoundly the whole character of the arc and the nature of the radiation emitted as a result of secondary collisions. One indication of this is the enormous lowering of the specific resistance of the arc, which makes possible a far wider gap than when the arc is struck between the electrodes alone. If metallic electrodes are used (both silver and copper were tried), this intensification of the lines due to impurities does not seem to take place at all, presumably because there is in any case an abundance of easily-ionised material, so that the presence of the sodium chloride makes comparatively little difference to the general character of the arc.

It may be mentioned that sodium sulphate produced exactly the same effect as sodium chloride; with calcium chloride the general tendency was the same, but the effect was not so clearly marked. Metallic silicates (Felspar and muscovite) brought about great intensification of the vanadium lines while pure silica had no effect. These facts would seem to indicate that the cations are

the essential agent, and that the phenomenon is due, not to the liberation into the arc of volatile compounds of the impurities, but rather to a change in the energy relations between the atoms of the impurities and those constituting the main part of the arc.

Since the ash of most tissues consists largely of salts of the alkalies and alkaline earths, it is clear that the failure to take account of this property of the graphite arc renders suspect many analyses of biological material that have used graphite as the only light source. Some authors do not mention at all the impurities in their electrodes; others have clearly assumed that comparison with the "blank" spectrum was a sufficient safeguard. Newell and M'Collum (1931), for example, used graphite electrodes which admittedly showed vanadium lines in the "blank" spectrum; these lines, however, did not include λ4460, so its presence or absence was used as a criterion of the presence or absence of vanadium in the specimen. It is not surprising that they report the presence of both vanadium and titanium in the majority of their samples. It is significant that all the reports of the presence in milk (whether cow's or human) of vanadium, titanium, and lithium (Wright and Papish, 1929; Zbinden, 1931; Blumberg and Rask, 1933) come from workers that have used graphite electrodes. Drea (1935), in his accounts of the elements in eggs and chick tissues, gives no information about impurities in the electrodes; but for some of the work, at any rate, graphite was used, and the universal appearance of vanadium and titanium in the analyses is suspicious.

Attention has so far been directed mainly to titanium, vanadium, and lithium, since the other three elements whose lines show the intensification effect—copper, iron, and calcium—are almost certainly universal in living tissues, so that, as far as qualitative analyses are concerned, their presence in the electrodes is a matter of indifference. But it is probable that different batches of graphite, especially if prepared by different manufacturers, will contain different impurities, and in order to be able to assess the reliability of the results of other workers it was necessary to ascertain which elements were liable to show the intensification effect, irrespective of whether they were present in the sample of graphite in which the effect was first noticed. Pieces of the electrodes were accordingly artificially contaminated by boiling and cooling them in solutions containing from 0·005 per cent. to 1 per cent. of the various elements, the strength being varied empirically in accordance with the spectroscopic sensitivity of the element in question in order that it should exhibit a few lines in the "blank" spectrum clearly but not too strongly. It must be understood that the results detailed below are applicable only when these conditions are observed; there appears to be for each element a maximal concentration above which no intensification of the spectrum takes place.

Thirteen elements, in addition to the nine mentioned in the spectrographic report on the electrodes, were studied. They were chosen so as to include all those commonly reported as present in biological material. The results are as follows:—

*Aluminium.*—λ3961 may appear though previously invisible, but λ3082 shows no intensification.

*Barium.*—λ5535 is intensified, as is also λ4554 if it is sufficiently weak; the other ionised persistent lines (λ4934 and λ2335) are not intensified.

*Boron.*—No intensification.

*Cadmium.*—No intensification of the very sensitive line λ2288, even at the greatest dilutions.

*Calcium.*—Marked intensification of λ4227, λ3968, and λ3934.

*Chromium.*—Very great intensification of all the persistent lines.

*Cobalt.*—No intensification.

*Copper.*—Marked intensification of the two very sensitive lines λ3248 and λ3274.

*Iron.*—Marked intensification of several lines, especially in the neighbourhood of λ2500.

*Lead.*—No intensification.

*Lithium.*—λ6707, though it never becomes very strong, may be made to appear, though invisible in the blank spectrum.

*Magnesium.*—No intensification of the two very sensitive lines near λ2800; the still more sensitive λ2852 is masked by sodium lines.

*Manganese.*—The triplet at λ4033, also λ2801 and λ2798, show a fair degree of intensification, but none of the ionised lines in the region of λ2600 are affected.

*Molybdenum.*—Very great intensification of all the persistent lines.

*Nickel.*—λ3414 shows a very slight degree of intensification.

*Silicon.*—No intensification.

*Strontium.*—The three persistent lines, especially λ4607, are unmistakably but not very strongly intensified.

*Silver.*—If the concentration is very weak the two persistent lines are considerably intensified.

*Tin.*—No intensification.

*Titanium.*—The spectrum of this element in the sodium-containing arc is never very strong, but it appears very regularly, though the element is only present to the extent of one part in five million of graphite, and is completely undetectable in the blank spectrum.

*Vanadium.*—All the principal lines, and especially the triplet at λ3184, are very strongly intensified.

*Zinc.*—No intensification.

It will be seen that the response of the elements is to some extent correlated with their position in the Periodic table. The majority of those whose lines are intensified under the conditions described belong to the transition series, and those in which the phenomenon is most strongly marked (Ti, V, Cr, Mo) to the early part of the series, *i.e.*, to the *a* division of groups IV, V, and VI. It must be emphasised, however, that these results refer only to the electrodes under conditions similar to those of the actual analyses, and that by varying

these conditions it is probable that the list of elements exhibiting this phenomenon could be restricted or extended.

It is clear then that great caution must be exercised if graphite electrodes are to be used in the qualitative analysis of biological samples; and it appears likely that the dangers in the analysis of mineral samples are just as great; for Papish and O'Leary (1931) have shown, apparently without realising that their results were directly applicable to impurities in the electrodes, that the intensity of the spectrum produced by a given weight of chromium is increased sixteen-fold by its incorporation with a large quantity of silica into a synthetic ruby. Quantitative analyses, though they may often be rendered impossible, are not so likely to give misleading results, since they depend, if properly conducted, on the comparison of the spectra of the standard and the specimen, photographed as far as possible under identical conditions.

The precautions necessary to avoid errors arising from the intensification effect are to take several photographs, with varying amounts of salt, time of exposure, etc., of the graphite arc into which has been introduced spectroscopically a pure salt of whatever element forms the greater part of the samples to be analysed, and then to compile a list of suspect elements from all those whose lines can be detected in any of these spectrograms. Information with regard to the presence of these elements in the specimens can only be obtained by using metallic electrodes as well. These precautions have been taken in all the analyses given in this paper and in subsequent papers of the same series. In addition, indications from the graphite arc of the presence of chromium or molybdenum have been ignored, unless at any rate a trace of their spectrum could be seen in the silver arc; for, although these elements are not mentioned in the manufacturers' report on the electrodes, and cannot be detected in any of the spectrograms of the "Specpure" sodium chloride, their extreme susceptibility to the intensification effect, coupled with the fact that the distribution of the impurities in graphite is often very irregular, makes such a precaution desirable.

## Summary.

A revised periodic tabulation of the biological elements is presented. The scope and limitations of the spectrographic method of analysis of biological material are discussed, and attention is drawn to a hitherto unrecognised but serious source of error arising from the effect of tissue salts in unmasking "latent" impurities in the graphite electrodes.

The activity of the organism as a geological agent is illustrated by analyses of peat ash at different depths.

The phenomenon of biological discrimination between different elements is illustrated by analyses of seed ash and the ash of the baker's and brewer's yeast.

The work has been carried out during the tenure of a Maintenance Allowance granted to D. A. Webb by the Ministry for Industry and Commerce of the Irish Free State. And the additional expense incurred has been defrayed by a bequest made to one of us (W. R. F.) by the late Edward Gordon Peake.

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## EXPLANATION OF PLATE 15.

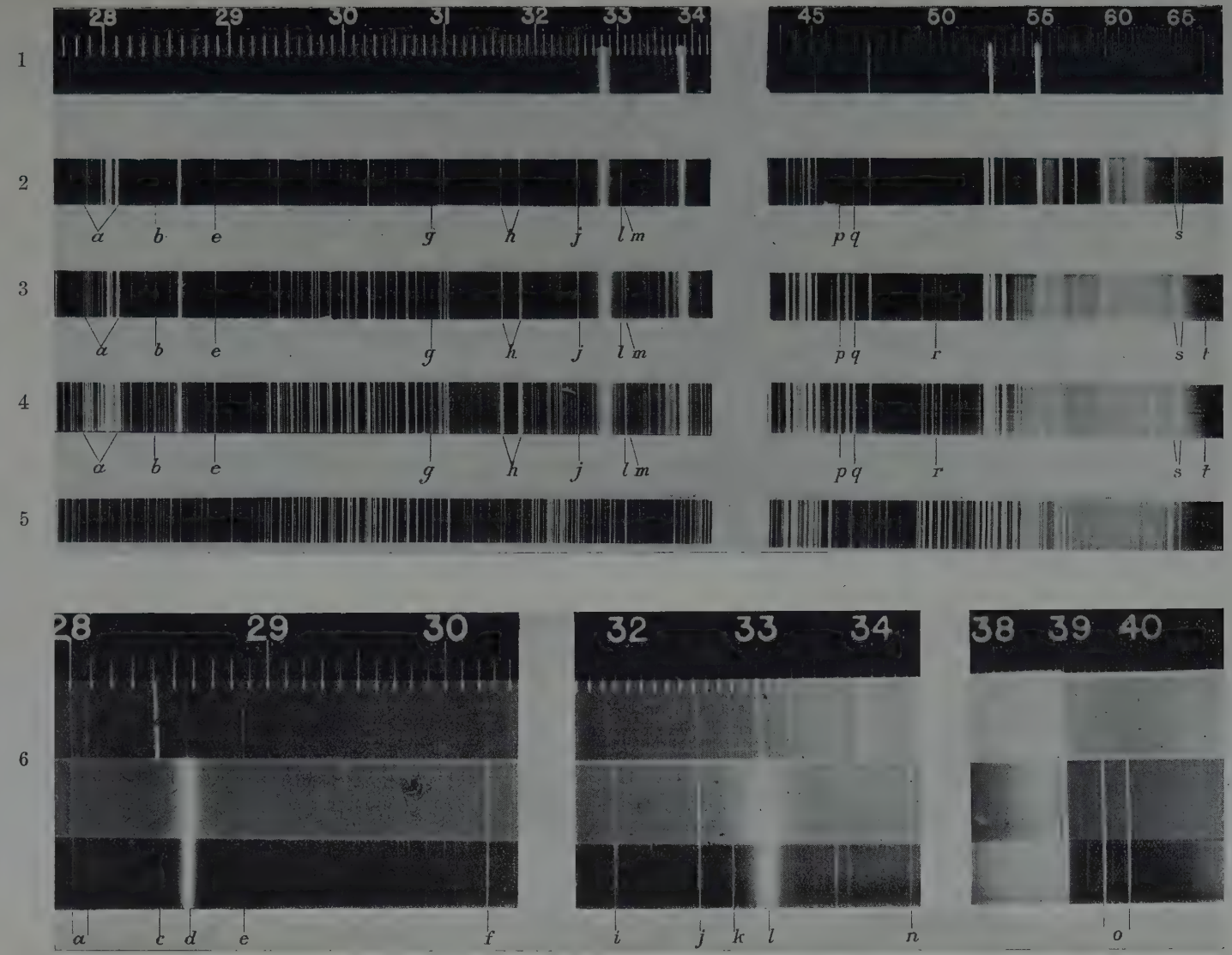
1 to 5 are enlargements of certain regions of the spectra of the three specimens of peat ash referred to in the text, together with the spectrum of the silver electrodes used and of the iron arc. Only those regions that exhibit characteristic lines of the elements actually met with have been reproduced. The presence of silver (which was detected by using graphite electrodes) cannot be demonstrated. The spectra are as follows:—

1. Blank spectrum of electrodes, with wave-length scale in units of 100 A.
2. Ash of peat from near the surface.
3. Ash of peat from a depth of 5 feet.
4. Ash of peat from a depth of 20 feet.
5. Iron arc.

Spectrum 6 illustrates the behaviour, referred to in the appendix, of certain of the impurities in graphite electrodes. The uppermost section of the triple spectrum is given by the electrodes alone; the lower two are produced by introducing into the graphite arc some spectroscopically pure sodium chloride. The consequent intensification of the lines of calcium, copper, vanadium, and iron is clearly visible.

The wave-lengths of the various lines marked on the plate and the elements responsible for their emission are given below.

*a* Mg 2777 to 2803.
*b* Pb 2833.
*c* C 2837 and 2838.
*d* Na 2853.
*e* Si 2882.
*f* Fe 3021.
*g* Al 3082.
*h* Ca 3159 and 3179.
*i* V 3183·4 to 3185·4.
*j* Cu 3247.
*k* Cu 3274.
*l* Na 3302 and 3303.
*m* Fe 3306.
*n* Fe 3437.
*o* Ca 3934 and 3968.
*p* Ba 4554.
*q* Sr 4607.
*r* Ba 4934.
*s* Ca 6439 to 6494.
*t* Li 6707.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No. 47.

# STUDIES ON THE ULTIMATE COMPOSITION OF BIOLOGICAL MATERIAL.

## Pt. II: SPECTROGRAPHIC ANALYSES OF MARINE INVERTEBRATES, WITH SPECIAL REFERENCE TO THE CHEMICAL COMPOSITION OF THEIR ENVIRONMENT.

By D. A. WEBB, B.A.

[Read April 20. Published separately May 29, 1937.]

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## I.—Introduction.

In an earlier communication (Webb and Fearon, 1937) the general aims and scope of this series of researches have been outlined, and the advantages and limitations of the spectrographic method discussed. The present paper contains a report on the elementary composition of the first series of specimens that has been subjected to systematic analysis. They consist principally of the tissues of marine invertebrates, but include also three species of algae, a fish, and a

terrestrial invertebrate (*Helix*). The algae were included in order to throw some light on the inter-relations between animals and plants with reference to chemical micro-constituents and their place in the food cycle. The fish serves to exemplify the differences in inorganic composition between vertebrates and the majority of invertebrates, while comparison of the snail with marine gastropods gives a measure of the chemical differences that may subsist between animals of the same class in accordance with different modes of life.

Several considerations led to the limitation of the survey mainly to marine invertebrates. In the first place it has become quite clear that the elementary composition of vertebrates is far more standardised than that of invertebrates, and that in the former the less familiar elements, even when clearly necessary for life, are found only in very minute quantities compared with those that sometimes obtain among lower animals. Furthermore, if an attempt is to be made, in considering the composition of the organism, to take into account the composition of the environment, it is clearly necessary to select only those organisms whose environment has a known and reasonably constant chemical composition. These conditions are fulfilled only by sea water. Finally, the fact that the chemistry of marine invertebrates is a very varied and, except for the preliminary survey by Fox and Ramage (1931), comparatively unexploited field renders the confirmation and extension of the work of these authors desirable.

The work has been carried out partly in the Physics Department, Trinity College, Dublin, and partly in the University Department of Zoology, Cambridge. I wish to express my indebtedness to Prof. W. R. Fearon for originally suggesting the research and for his constant interest in its progress; to Prof. R. W. Ditchburn for his unsparing help and advice on the physical aspects of spectrography, and to Dr. C. B. Allsopp, who performed the same services for me at Cambridge; and to Prof. L. P. Renouf, of University College, Cork, who has done much to facilitate the collecting of specimens at Lough Ine. My thanks are also due to the Board of Trinity College for a research prize from the John Winthrop Hackett Fund, and to the Department of Education of Saorstát Éireann for a maintenance grant; during the tenure of these the earlier part of the work was done.

## II.—Material and Methods.

(*a*) *Preparation of material.*—Individual organs when required were dissected out from living animals, narcotised if necessary. The specimens were if possible dried and ignited immediately, but in some cases were preserved in alcohol, the fluid being evaporated down and ignited with the specimen. If reasonably pure alcohol is used there is no danger of contamination. If a whole animal was to be analysed it was, if circumstances permitted, starved for several days before being killed, in order to eliminate as much as possible of the foreign matter in its gut, gills, or other organs. In spite of this precaution, accompanied of course by a careful washing of the surface in sea water, some specimens gave

an ash whose silicon and aluminium content was so high that it was obvious that there was a considerable degree of contamination with mineral particles. Analyses of these specimens must be interpreted with great caution, but they are by no means entirely worthless. They provide at least negative evidence with regard to some elements, and since the general composition of marine muds is tolerably constant it is easy to compile a list of elements which, if found in the ash, may be ascribed with reasonable certainty to the animal and not to the detritus. The dangers of this type of contamination, and their apparent neglect by most previous workers, have been discussed in the first paper of this series.

The samples were ignited over a Bunsen burner in a silica crucible, having been in most cases first dried at 100° C. This method gave entirely satisfactory results, and the use of a muffle seems quite unnecessary. Provided the flame is not allowed to play directly on the specimen there is no reason to suppose that contamination with the material of the burner can possibly occur. Complete ignition to a white ash is difficult to achieve, but it was soon found that it is sufficient to burn off all the volatile organic compounds so as to leave a "clinker." If this has been accomplished, the presence of quite a large amount of carbon in the ash can be tolerated without sacrificing the clarity of the spectra. This means that intense and prolonged heating, which is liable to volatilise certain constituents of the ash, and which often results in converting it to a glassy slag which adheres tenaciously to the crucible, can be avoided. Since the method of quantitative estimation adopted relies on establishing the ratios existing between the various metallic components of the ash, the quantity of carbon that remains, as well as the nature of the acid radicles in the ash, is a matter of indifference, nor is it necessary for the ash or the tissue itself to be weighed.

The incineration of specimens with a high fat content, or with a particularly high ratio of organic to inorganic material, was accelerated by the addition of two or three drops of pure nitric acid.

As soon as the inner surface of a crucible became rough to the touch it was rejected, since it thereby became more difficult to clean, and also the danger of flakes of silica becoming incorporated in the ash was increased.

In order to reduce all samples to a common anionic basis the ash was ground up in an agate mortar with an equal quantity of Hilger's "Specpure" ammonium sulphate, as recommended by Lewis (1932). Since the different ashes contained a varying amount of carbon it was impossible to ensure a constant ratio of ash to ammonium sulphate, but there seems to be little advantage in so doing. Attempts were made to compress the mixture into pellets of standard weight, but this practice was soon abandoned when it was found that all the spectra showed strong evidence of contamination from the metal of the block sold for the purpose. The loose powder was therefore placed on the lower electrode of the arc without weighing. The employment of an exactly equal weight of ash for each exposure does little to increase the accuracy of the results, since the quantitative estimation must in any case rely

not on absolute intensities but on relative intensities of the lines of the different elements within the same spectrogram.

(*b*) *Description of apparatus.*—A Hilger medium quartz spectrograph was used. The two instruments employed at Dublin and Cambridge, respectively, though not identical models, possess essentially the same optical system, and the difference in dispersive power does not exceed 1 per cent., so that spectrograms taken on one could be safely compared with those taken on the other. Several specimens were photographed on both instruments in order to establish the safety of this procedure. This size of spectrograph is the most suitable for general purposes, since it enables the whole spectrum to be taken on one plate, and at the same time has a dispersion sufficient to resolve almost all pairs or groups of lines that are likely in practice to cause difficulty. The plates used were Ilford's rapid process panchromatic, which have a considerable and fairly uniform sensitivity from 6700[1] to 2300. A quartz spherocylindrical lens was used to focus the light on the slit; this permits of considerably shorter exposures, which is in many ways an advantage, and the objection to the use of such a lens in quantitative work does not seem to be well founded.

(*c*) *Choice of electrodes.*[2]—Carbon, graphite, copper, and silver electrodes have all been tried; each material possesses its own defects and advantages. The dangers inherent in the use of carbon and graphite have already been discussed (Webb and Fearon, 1937; Webb, 1937). Graphite is on the whole preferable to carbon, since its impurities (mainly non-metals and semi-metals) are less objectionable, and it is considerably cheaper. Copper is in many respects very satisfactory, but was eventually rejected in favour of silver, which, although rather costly, has the following points in its favour:—

(1) It can be obtained in a remarkably high degree of purity, and in particular is completely free from iron, which seems to be present in traces in even the purest samples of copper.

(2) Clearly copper cannot be searched for with copper electrodes, nor silver with silver electrodes. It is better to sacrifice silver, both because it is less constant in biological material than copper, and usually of less interest, and also because silver can be detected in the graphite arc, while copper, being a constant impurity whose lines are subject to intensification, cannot.

Although a complete analysis for all elements except silver can be made with the silver arc, the specimens were subjected to analysis in the graphite arc as well, for it was found that, owing probably to its higher temperature, the latter is able to disclose quantities of several elements which escape detection in the former. This applies especially to nickel, lead, and manganese. The whole question of the variation of arc sensitivity of elements in accordance with the composition of the electrodes demands further investigation, since it should,

[1] Wave lengths are given throughout in Ångström units.

[2] All electrodes referred to are Hilger's H.S.

if only a single element is under consideration, be an important factor in determining the choice of electrodes.

(*d*) *Details of procedure.*—An ordinary arc stand was used, with the usual arrangement for symmetrical approximation and separation of the points. The graphite electrodes were cut up into lengths of about 15 mm., which were held by steel holders. Fresh pieces were used for each specimen, attempts to clean them after use by boiling in acid and heating to dull redness having proved unsuccessful. The silver electrodes were cleaned by filing down to a depth of about a millimetre, pickling for a few moments in 20 per cent. nitric acid, rinsing in distilled water, and drying with filter paper. In both cases the lower electrode was flat, the upper turned to a blunt point. The ash was simply placed with a stainless knife or spatula on the lower electrode in sufficient quantity to cover its surface thinly, and the arc struck by bringing the points together. Most authors recommend the drilling of a cavity in the lower electrode to receive the powder; this was tried, but did not seem to produce burning conditions that were more constant or in any way more reliable. In any case it would be impossible for the silver electrodes without very great labour and waste of material.

Three exposures of varying length were taken of each specimen with each type of electrodes. By this variation in exposure time the sensitivity of the method is increased, for under-exposed spectrograms will often show up a line that is apt to be obscured by bands or halation from a neighbouring strong line, while those that are over-exposed may contain lines that are otherwise too faint to be seen. It also facilitates the quantitative estimations as will be shown later.

By means of a Hartmann three-aperture diaphragm the blank spectrum of the electrodes and the spectrum of the iron arc were photographed in juxtaposition to the spectrogram of the specimen. The use of the iron arc enables almost all lines to be determined with certainty without the labour of measurement. It is, of course, essential that the plate-holder should not be moved between the photographing of the specimen and of the iron arc, since a lateral displacement of less than 0·05 mm. between the two spectra is sufficient to make this method useless for reading off wave-lengths, and even on the best instruments the plate-holder is liable to this amount of displacement in the course of its vertical travel.

The spectra were disposed on the plate thus:—

Electrodes (blank).
Specimen (10 secs.).
Iron arc.

Specimen (5 secs.).
Iron arc.
Specimen (15 secs.).

A narrow slit (about ·03 mm.) was used since microphotometric measurements were not required.

## III.—Assessment of Results.

(*a*) *General considerations on quantitative spectrography.*—It has already been pointed out that, on the one hand, a purely qualitative study of the elements found in tissues yields little or no useful information, but that, on the other hand, a wide margin of error can be tolerated in the application of quantitative methods, provided, of course, that it is recognised. Until a few years ago it was generally believed that any attempt to draw quantitative conclusions from spectroscopic data was doomed to failure on account of the numerous factors which were able to cause variations in the strength of any given line. But the matter has recently been subjected to intensive study by several workers, and the reliability of their methods, within the limits of error indicated, is no longer open to doubt. Accuracies as great as 5 per cent. are sometimes claimed, but it is probable that these can only be obtained in certain specially favourable cases, which are in any case amenable to chemical analysis.

All quantitative methods rest on the fundamental postulate that the number and intensity of the lines of an element bear a direct relation to its concentration in the light source. They are, however, clearly divisible into two groups:

(1) Those that rely on producing exactly the same conditions (thermal, electrical, etc.), and giving exactly the same time of exposure in the photography of specimens and standards.

(2) Those that establish concentrational ratios between two components by the use of some kind of internal standard principle.

The dangers of the first type are obvious, and, although it may be applicable to flame spectra (the method employed by Ramage rests on this principle), and perhaps to spark spectra, when the material to be examined is of very constant composition and physical properties, the impossibility of ensuring any constancy in the degree or quality of excitation in the arc renders its use for this type of light source quite impossible.

(*b*) *Gerlach's method of homologous lines.*—This method, of which details are given by Gerlach and Schweitzer (1931), pp. 67–94, seems in many respects to be the most trustworthy of all quantitative methods, but it was devised primarily for the analysis of alloys, and cannot be applied directly to biological samples, whose principal constituents (alkalies and alkaline earths) are unable, because of the simplicity of their spectra, to provide an adequate supply of homologous pairs. As a means of surmounting this difficulty the addition of some element, such as cobalt, to the specimens has been suggested, thereby providing an intermediate standard, whose spectrum is rich in lines of various degrees of intensity. This would, however, preclude the detection of cobalt in the specimen, and though it would be possible to find some other element suitable for an auxiliary standard—*e.g.*, platinum—that very property which renders it suitable for the purpose, namely, the complexity of its spectrum, makes one unwilling to introduce it into the already complex spectrum of a biological ash. In any case there are few pairs of elements for which tables

of homologous pairs and the ratios to which they correspond have been published, and the number of standards that would have to be prepared to draw up such tables for all the elements liable to occur in living tissues is immense.

(*c*) *Lewis' ratio quantitative system.*—The underlying principles of this method (Lewis, 1932) are those actually employed in the present work, though considerable modifications in detail were necessary, the method as described being suited chiefly to routine examination of comparatively invariable material. The essential difference between this and Gerlach's method may be seen by considering the case of a mixture of two elements *A* and *B* in unknown proportions. In Gerlach's method a search is made for two neighbouring lines $\Lambda_A$ and $\Lambda_B$, which satisfy the criteria necessary for a homologous pair, and are, in the spectrogram of the specimen, equal in intensity. Reference is then made to tables or to the spectra of standards of known composition to ascertain what proportion of *A* to *B* produces the equivalence of this pair of lines. By Lewis' method a search is made among the spectrograms of a graded series of standards till one is found which satisfies the condition that if $\lambda_a$ in the standard is equal in intensity to $\lambda_a$ in the specimen then $\lambda_b$ will also be of equal intensity in the two spectra. The ratio of *A* to *B* is then the same in the specimen as in the standard.

One of the advantages of this system over the method of homologous lines is that it permits of greater elasticity. The pair of lines selected for comparison need not be close together, nor need they be equal in intensity within the range of concentrations studied, though the more closely both these conditions are fulfilled the more accurate are the results likely to be. Furthermore, several lines can be used to establish any given concentration of an element.

In two respects a departure was made from Lewis' scheme. First, it was not found necessary to establish by absolute means the percentage of one of the dominant elements; the determination of ratios is sufficient, as will be explained later. Secondly, no attempt was made to synthesise a separate standard in imitation of each specimen. This is possible in routine industrial or agricultural analyses of numerous samples of the same type of material, in which only a few elements are being studied; but in the material here under discussion the variation both in the proportions of the major constituents and in the variety of the minor constituents would render the task endless. It was, therefore, assumed that if the *general* composition of the standard with regard to major constituents was the same as that of the specimen, equal intensities of the spectrum of a minor constituent of each would represent equal concentrations, even if the concentrations of the other minor constituents differed fairly widely in the two. (By minor constituent is implied a concentration not exceeding 3 per cent.) To what extent this assumption is justifiable must remain doubtful, since our knowledge of the effect of the presence of one element on the behaviour of another in the arc is still extremely limited. Nevertheless, some experiments of Gerlach and Schweitzer (1931, pp. 71–72) indicate that a homologous pair of lines preserves the same relative sensitivity

under a very wide variety of conditions, so that it is unlikely that any considerable error arises from this cause. It must be pointed out that the intensification effect of the lines of impurities in graphite electrodes depends on a very fundamental change in the character of the arc, far greater than any difference that could be brought about by the variation in the composition of different biological ashes.

(*d*) *Preparation of the standards.*—The ash of marine invertebrates consists almost entirely of salts of sodium, potassium, magnesium, and calcium. The proportions between the metals vary widely, but they often approximate to some extent to those found in sea water. It was decided, therefore, to make sea water the basis on which the standards were to be made up. Since the proportions of these principal constituents are constant, and the minor constituents are few and present in known concentrations, it serves the purpose admirably, and saves the expense of purchasing the very pure salts that would be necessary if the standards were made up entirely by synthesis. Two different samples of sea water were used at different periods of the work—one from Sandycove, on the south side of Dublin Bay, the other off-shore water from the English channel near Plymouth. No difference in composition between the two could be detected spectroscopically.

The standards were made up by evaporating 25 c.c. of sea water, to which the required quantities of other elements had been added in the form of solutions, and grinding up with ammonium sulphate. Two series were required.

(1) Those consisting of sea water in which the ratio of the major constituents remained unaltered, but to which minor constituents had been added, all in equal quantities by weight. The concentrations selected were 3, 1, 0·3, . . . 0·001 per cent. of each minor constituent, expressed in terms of the total weight of *cations* present.[3]

(2) Those to which no minor constituents had been added, but in which the proportions of the major constituents had been altered by the addition of spectroscopically pure salts of sodium, potassium, calcium, or magnesium. As in the other series, the interval between two successive ratios was $\sqrt{10}$. The range covered was:—Na/K, from 100 to 0·1; Na/Mg, from 100 to 0·3; Na/Ca, from 300 to 1; Ca/Mg from 10 to 0·01.

The first series of standards was photographed on both silver and graphite electrodes, the second on silver alone. In all cases a considerable number of exposures was made, varying widely in length, so as to ensure that one at least would be of a suitable degree of intensity to compare with one of the spectrograms of each specimen.

(*e*) *Choice of lines.*—The accuracy of the method is very largely dependent on the judicious choice of the lines to be compared. In general, the criteria

[3] It is an essential feature of the ratio quantitative system, and indeed of all systems that make use of the internal standard principle, that in calculations of ratios and percentages the anions are ignored.

recommended by Gerlach for the establishment of a homologous pair have been adopted, except for the provision that recommends that the pair of lines shall be spectroscopically adjacent. The fulfilment of this condition is of importance if the results so established are to be extended to different types of spectrographs or plates, or if, as in Gerlach's method, equality of intensity is to be gauged between two lines in the same spectrogram; but in the method here described, where the comparison is made, for both members of the pair, from one spectrogram to another it is scarcely necessary. But care was taken to comply with the other conditions—namely, that the lines should have an intensity lying within the range of correct exposure, thus ensuring that their relative sensitivity ($\delta I/\delta C$) is maximal, and that an ionised line of one element should never be used in conjunction with a neutral line of another, since the intensity ratio of neutral to ionised spectra is very easily altered by variation in the electrical conditions. The nature of the lines was determined from Kayser's *Hauptlinien* and the *International Critical Tables*, but in neither are the necessary data given for tin or lead. In the case of these elements, therefore, consistency of results was held to indicate that appropriate lines had been chosen.

(*f*) *Making the comparison.*—The plates were examined in a double comparator, which enabled the corresponding lines to be apposed in the same field. The ratios of the four primary constituents (Na, K, Mg, Ca) were first determined. Assuming that they constituted at least 90 per cent. of the metals of every ash examined, it follows that, if the ratio of a minor constituent to any one of the major constituents is determined, its concentration relative to the total metal content can be calculated.

While it has, for the sake of simplicity, been assumed in the foregoing discussion that only a single line of each element is used in establishing a ratio, in practice several lines of the major constituent, and if possible of the minor also, were scrutinised to establish the equivalence of the two spectra.

It must be mentioned that sea water, besides the four major constituents already referred to, contains five other elements that can be detected spectroscopically (Li, Cu, Ag, Sr, B). Of these, the three first lie near the limit of visibility, and are present in concentrations of not more than 0·0001 per cent. of the total weight of metals present, so that they did not interfere with the standards. Allowance was made for the boron and strontium content, their amounts being known.

Interpolation was, of course, necessary between the values represented by the standards, and though one becomes fairly adept at this with practice, it probably represents a source of error large enough to cover all others that may be present in the technique. For a minor constituent deviations from the true value of anything up to 70 per cent. in an upward and 40 per cent. in a downward direction must be expected. For a major constituent the error is probably rather less. As will be seen from the results, the range of variation is so great that even a margin of error of this size leaves plenty of room for significant conclusions to be drawn.

## IV.—Sensitivity and Reliability of the Method for the Various Elements.

The lowest concentration that can be detected varies considerably from one element to another, depending as it does, primarily on the "arc sensitivity" of the element itself, but also on the nature and purity of the electrodes and the presence or absence of other elements that may cause interference with its most sensitive lines. For a proper understanding of the analyses it is necessary to realise not only the limits of sensitivity for each element, but also the factors that determine these limits, since they are often different in specimens of different composition.

All elements detected in one or more of the specimens are discussed below from this point of view. Elements that were regularly searched for, but did not occur in detectable quantities, are—*antimony, arsenic, beryllium, germanium, gold, mercury,* and *titanium.* Of these, arsenic and titanium might have been expected to occur; but the former has an extremely low arc sensitivity, and the latter could only be searched for with silver electrodes, on which it cannot be detected in concentrations of less than 0·1 per cent. of the metals. Many other elements, such as the metals of the palladium and platinum groups and the less familiar earths and transition elements, can also be detected by the arc, but in view of the extreme unlikeliness of their occurrence they were not regularly searched for.

*Lithium.*—Occurs in the graphite as an impurity liable to intensification. On silver only 6708 was ever shown; this lies so far into the red that the sensitivity of the plate is extremely variable; on some plates sea water showed the line quite strongly, but on others scarcely at all. It was decided, therefore, that quantitative measurements were impossible, and only presence or absence is recorded.

*Sodium.*—The limit of sensitivity is never even approached. The doublet at 2680 is generally the most useful for comparison.

*Potassium.*—Always detectable, and usually with two doublets available for comparison. Some trouble may be experienced if the Fe is high and the K low, as K 4047·2 may be fogged by Fe 4045·8, but this seldom happens.

*Rubidium and Caesium.*—No data are presented for these elements, as their *raies ultimes* lie in the infra-red, and of the lines in the visible region Rb 4215·6 coincides with Sr 4215·5, which is always present; while Rb 4201·8 and Cs 4555·3 coincide with lines of iron and barium respectively, which are always liable to be present. In a few cases a probable indication of rubidium was seen, but the results are too uncertain to be worth while reporting.

*Magnesium and Calcium.*—The limit of sensitivity is never approached, and suitable lines for comparison are plentiful. The ionised lines, Ca II 3158·9 and 3179·3, are useful when only an ionised line of a minor constituent is available for comparison.

*Strontium.*—Detectable to 0·005 per cent. of the cations or less, and always present in concentrations exceeding this. 4607 can be used with neutral lines, 4078 with ionised. 4215·5 is generally fogged by Ag 4212.

*Barium.*—Detectable to 0·002 per cent. All the lines suitable for comparison are ionised. Silver electrodes are more reliable for detecting small traces, since the most sensitive line (4554) coincides with a cyanogen band.

*Boron.*—The arc spectrum consists only of one doublet, which can be seen in concentrations down to about 0·01 per cent.

*Aluminium.*—The results with graphite are unreliable. In the silver arc 3082 seems to be as sensitive as the lines usually considered to be the *raies ultimes*. It lies in a region subject to bands, but with a narrow slit and good magnification can be seen to lie between the flutings. The limit of sensitivity is 0·03 per cent.

*Silicon.*—Unfortunately this element is present as a constant impurity in graphite, which, although it is not subject to intensification, prevents the detection of small traces. On the other hand, its sensitivity in the silver arc is low, the limit being about 0·25 per cent. 2882 is a very reliable line, and not subject to interference.

*Vanadium.*—The results on graphite are entirely worthless. In the silver arc the triplet round 3184 can be seen down to 0·02 per cent.

*Chromium and Molybdenum.*—Although these elements are not mentioned in the manufacturers' report as being present in the graphite, their occasional occurrence in graphite spectrograms of specimens whose silver spectrograms do not contain them renders it probable that they do occur discontinuously in the electrodes in minute quantities. It may be recalled that they are extremely susceptible to the intensification effect. Accordingly, neither element was recorded as present unless it was detectable in the silver arc. The limits of sensitivity for the lines Cr 4254 and Mo 3133 are 0·002 and 0·004 per cent. respectively.

*Manganese.*—Good corroboration can be obtained by comparing the results in both types of arc, for with graphite the ionised line 2476 is the most sensitive, but with silver the neutral line 4031. 2798, lying between the two strong magnesium lines, is often the most useful for comparison.

*Iron.*—The limit of sensitivity (about 0·02 per cent.) is set by the inevitable contamination that results from the use of an iron file to clean the silver electrodes and from the escape of particles from the iron arc into the atmosphere. If the element is at all abundant there is a great variety of lines available for comparison.

*Nickel and Cobalt.*—Both are best detected in the graphite arc. The limits are 0·01 and 0·015 per cent. respectively.

*Copper.*—Always present in the graphite electrodes, and sometimes in the silver, if the cleaning has been carelessly done. 3274 is nearly always obscured by halation from Ag 3281, so that 3248 is usually the only line available for comparison. As it is extremely subject to reversal it is not very satisfactory. In specimens very rich in copper 2492 is useful. The limit of sensitivity is about 0·0005 per cent.

*Silver.*—In the graphite arc the two *raies ultimes* are extremely sensitive, and, in the concentrations in which the element is liable to occur, of suitable intensity. The results for this element are, however, incomplete, since specimens photographed soon after the silver arc has been struck in the same room show distinct evidence of contamination from the atmosphere. The limit of sensitivity is about the same as for copper, or somewhat lower.

*Zinc.*—This is an element for which it is extremely difficult to obtain satisfactory results. Its arc sensitivity is low, and, of the most sensitive lines, 4810·5 and 4722·2 coincide with Sr 4811·9 and 4722·3,[4] which are always liable to be present though faint, 4680 coincides with a faint silver line, and in any case falls in a region in which the sensitivity of the plate is somewhat variable, 3302·9 and 3302·6 are masked by the sodium doublet, and 3282 is hidden by halation from Ag 3281, though it can be used in graphite exposures. There remain only the very sensitive 2138·5, which requires special sensitisation of the plate, a procedure incompatible with quantitative results, and 3345·0 + 3345·5, the doublet that was actually used. Even this may be partly obscured by the very diffuse line Ca 3344·5 if the amount of calcium present is large. The limit of sensitivity cannot be placed below 0·2 per cent., and for highly calcareous samples is even higher.

*Cadmium.*—The line 2288 is extremely sensitive, suitable for comparison, and quite reliable in the absence of As 2350. Concentrations down to 0·001 per cent. can be detected.

*Tin.*—2840 and 2863·3 were used. Limit of sensitivity 0·01 per cent.

*Lead.*—2833 could always be used, and is visible down to 0·003 per cent.

*Phosphorus.*—The arc sensitivity, though higher on graphite than on silver, is very low on both. No accurate quantitative data were recorded, but probably 1 per cent. is the lowest concentration that can be detected.

*Fluorine.*—Calcium fluoride gives rise to a well-defined band head at 5291, from which the presence of fluorine in the specimen may be inferred, though there is no reason to suppose that it is present as calcium fluoride even in the ash. The intensity of the band is probably affected by the amount of available calcium as well as the amount of fluorine, and it is possible that a considerable quantity of fluorine might escape detection if the calcium content were low. For this reason the limit of sensitivity is necessarily vague, but it appears to be of the order of 0·5 per cent. of fluorine.

## V.—Results.

The results of the analyses are for the most part presented in Table I, though a certain amount of additional data incapable of precise quantitative expression is given in the discussion below. A word is necessary with regard to the units in which the concentrations of the various elements are expressed.

[4] This pair of coincidences, which is overlooked by Gerlach and Gerlach (1934, p. 114), is pointed out by Swings (1935, p. 80).

On reading through the literature of tissue analysis, one finds that some authors calculate the percentages in terms of fresh weight, others in terms of dry weight, and others again in terms of ash. Any idea of obtaining results that will be directly comparable with all previous analyses is thus ruled out from the start. Nor is it clear which of these methods of expression gives the most valuable information. Dry weight is usually accepted as the most significant physiologically, but when ratios of inorganic components are being considered ash weight is probably more relevant, while for ionic constituents only the wet weight gives an idea of the degree of dilution. It was decided therefore to present the figures without further recalculation in the form in which they came to hand, *i.e.*, as percentages of the total weight of cations in the ash. This is in any case a more absolute measure than percentages of ash weight, since the proportions of chlorides, carbonates, oxides, etc., will vary considerably with the conditions of incineration. The division factors necessary to convert these figures to the more familiar modes of expression vary greatly with the type of tissue, but for the soft tissues of a marine invertebrate it can be assumed that the factors are approximately 2·5, 7, and 50, for ash, dry, and wet weight, respectively. For tissues that are partly skeletal the second and third factors will be considerably lower.

*The alkalies.*—No comment is required on the distribution of sodium and potassium. The Na/K ratio is, as might be expected, fairly closely correlated with the water content, and particularly low in the algae.

It has been already explained why quantitative data for lithium are not available. It may, however, be stated with some confidence that in *very few, if any, of the specimens examined is the concentration of lithium higher than in their environment.* The concentration in sea water has been estimated spectroscopically by Thomas and Thompson (1933) as 100 mg. per cubic metre,[5] or 0·00075 per cent. of the cations. Bardet *et al.* (1937), using a chemical method of separation with spectroscopic checks on all the precipitates, obtained a somewhat higher figure (170 mg. per cu. m.).

The ease with which lithium can be detected by visual spectroscopy has led to several records of its presence in plant and animal tissues, but they are mostly only qualitative. Ramage[6] finds that it is much less generally distributed in man and other terrestrial or freshwater animals than in marine animals, but the geochemical evidence is insufficient to say whether this is correlated with its availability in the environment. He claims that in the latter it occurs in concentrations up to 0·005 per cent. of the dry weight; I am quite

[5] Cooper (1933) has suggested that minor constituents of sea water should be expressed in terms of milligram-atoms per cubic metre, and the use of this unit might well be extended to the micro-constituents of organisms. It was felt however that in the present paper the difficulties of correlating the results with those of other workers were already sufficiently great without the introduction of atomic weights as a fresh complicating factor.

[6] Reference to this author without further qualification indicates one or both of the papers by Fox and Ramage (1931) and Sheldon and Ramage (1931).

| Species | Organ | Locality | Number of individuals in sample | Li | Na | K | Mg | Ca | Sr | Ba | B | Al |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GASTROPODA | | | | | | | | | | | | |
| *Helix aspersa* | All soft parts | Dublin | 1 | (+) | 5 | 8 | 8 | 80 | 0·2 | 0·25 | 0·25 | 0·25 |
| *Littorina littorea* | ,, | Sandycove | several | + | 35 | 18 | 35 | 8 | 0·04 | — | 0·03 | 0·8 |
| *Nucella* (= *Purpura*) *lapillus* | ,, | ,, | 5 | + | 35 | 15 | 35 | 10 | 0·04 | — | 0·03 | 0·1 |
| *Pleurobranchus plumula* | Entire | L. Ine | 1 | (+) | 15 | 3 | 30 | 55 | 0·8 | 0·004 | 0·07 | 0·1 |
| *Aeolidia papillosa* | ,, | ,, | 3 | (+) | 75 | 15 | 8 | 2 | 0·02 | — | 0·03 | — |
| *Archidoris britannica* | Mantle | ,, | 3 | (+) | 25 | 2 | 20 | 55 | 2 | 0·008 | 0·01 | — |
| ,, ,, | Remainder | ,, | 3 | ± | 55 | 20 | 10 | 15 | 0·1 | 0·006 | — | 0·2 |
| LAMELLIBRANCHIATA | | | | | | | | | | | | |
| *Modiolus modiolus* | Gonad | Salcombe | 4 | (+) | 40 | 40 | 15 | 5 | 0·03 | 0·003 | 0·04 | — |
| *Pecten maximus* | ,, | ,, | 3 | — | 35 | 45 | 15 | 5 | 0·02 | — | | |
| ,, ,, | Mantle | ,, | 3 | (+) | 70 | 25 | 3 | 1·5 | 0·03 | 0·05 | 0·03 | — |
| ECHINODERMATA | | | | | | | | | | | | |
| *Marthasterias glacialis* | Entire | L. Ine | 2 | ± | 10 | 2 | 20 | 65 | 0 6 | 0·004 | 0·03 | — |
| *Asterias rubens* | ,, | ,, | 4 | + | 25 | 3 | 10 | 60 | 0·8 | 0·003 | 0·15 | — |
| *Ophiocomina nigra* | Disc, without arms or jaws | ,, | 4 | (+) | 7 | 7 | 20 | 65 | 1 | — | 0·08 | — |
| *Paracentrotus lividus* | Ovary | ,, | 6 | ± | 35 | 35 | 15 | 15 | 0·05 | — | 0·01 | 0·08 |
| ,, ,, | Coelomic fluid | ,, | 10 | + | 85 | 3 | 10 | 3 | 0·1 | — | 0·03 | — |
| *Echinus esculentus* | ,, | ,, | 2 | + | 85 | 3 | 10 | 3 | 0·1 | — | 0·03 | — |
| PISCES | | | | | | | | | | | | |
| *Lepadogaster gouani* | Entire | ,, | 1 | + | 15 | 45 | 3 | 40 | 0·15 | ± | 0·01 | — |
| UROCHORDATA | | | | | | | | | | | | |
| *Ascidiella aspersa* | Branchial sac. | ,, | 6 | (+) | 60 | 7 | 15 | 15 | 0·2 | — | — | 0·2 |
| CRUSTACEA | | | | | | | | | | | | |
| *Eupagurus bernhardus* | Muscle of chela | Salcombe | 1 | — | 25 | 12 | 10 | 50 | 1 | 0·005 | 0·01 | 0·08 |
| NEMERTEA | | | | | | | | | | | | |
| *Lineus longissimus* | Entire | L. Ine | 3 | + | 50 | 50 | 2 | 1 | 0·015 | 0·005 | 0·05 | 0·15 |
| POLYCHAETA | | | | | | | | | | | | |
| *Nephthys* sp. | ,, | Salcombe | 4 | — | 40 | 25 | 8 | 25 | 0 05 | — | 0·1 | — |
| ALGAE | | | | | | | | | | | | |
| *Ulva lactuca* | Frond | L. Ine | several | ++ | 45 | 45 | 7 | 3 | 0·02 | 0·02 | 0·06 | 0·3 |
| *Fucus serratus* | Receptacles | Sandycove | ,, | (+) | 40 | 55 | 2 | 2 | 0·2 | 0·03 | 0·06 | 0 1 |
| *Saccorhiza bulbosa* | Stipe | L. Ine | 1 | ± | 30 | 70 | 1 | 1 | 0·1 | — | — | — |
| Limit of sensitivity | ... | ... | ... | variable | ... | ... | ... | ... | ... | 0·002 | 0·01 | 0 03 |
| Sea water (average values) | ... | ... | ... | 0·0008 | 84 | 3 | 10 | 3·3 | 0·1 | <0·0015 | 0·035 | 0·01(?) |

Numerical data are expressed as percentages of the total weight of metals (cations) in the ash. In cases where numerical data cannot be given arbitrary signs are employed; ± indicates a doubtful trace, little greater than the limit of sensitivity, or perhaps absent, (+) a faint but definite indication, + a stronger indication, and so on. A dash — in all cases means a value below the limit of sensitivity.

| Si | V | Cr | Mn | Fe | Co | Ni | Cu | Zn | Cd | Sn | Pb | P | F |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| + | — | — | 0·08 | 0·8 | — | — | 0·6 | ± | 0·03 | 0·025 | 0·2 | ++ | — |
| ++ | — | — | 0·08 | 1 | — | 0·02 | 0·6 | — | 0·001 | — | 0·03 | ++ | — |
| ++ | — | — | 0·06 | 1 | — | ± | 0·6 | 2·5 | 0·03 | — | 0·1 | ++ | — |
| + | 0·1 | — | 0·015 | 1 | 0·025 | 0·15 | 0·02 | 1 | 0·04 | — | 0·02 | + | — |
| — | — | — | 0·04 | 0·7 | — | — | 0·015 | 1·5 | — | 0·15 | 0·03 | ++ | — |
| — | — | — | 0·02 | — | — | — | 0·01 | — | 0·03 | — | ± | + | ⩾ 2 |
| + | — | — | 0·07 | 1 | — | — | 0·03 | — | 0·01 | — | — | (+) | ⩾ ■ |
| — | — | — | 0·025 | 0·25 | — | — | 0·008 | — | — | — | 0·015 | +++ | — |
| | — | | | | — | — | | c. 3 | | — | | +++ | — |
| (+) | — | — | 0·008 | 0·4 | — | — | 0·007 | 0·6 | — | — | 0·02 | + | — |
| (+) | — | — | 0·01 | 0·05 | — | — | 0·01 | — | — | — | — | (+) | ± |
| + | — | — | 0·003 | 0·08 | — | — | 0·03 | — | 0·003 | 0·08 | 0·005 | (+) | — |
| (+) | — | — | 0·03 | 0·25 | — | — | 0·04 | — | 0·003 | — | — | — | + |
| (+) | — | 0·003 | 0·008 | 0·05 | — | — | 0·005 | — | 0·003 | 0·08 | ± | +++ | — |
| — | — | — | — | — | — | — | 0·003 | — | — | — | — | — | — |
| — | — | — | — | — | — | — | 0·008 | — | — | — | — | — | — |
| — | — | — | 0·015 | 0·15 | — | — | 0·04 | ± | — | — | 0·004 | +++ | — |
| + | >1 | — | 0·005 | 0·15 | — | — | 0·01 | — | — | — | 0·015 | — | — |
| (+) | — | — | 0·02 | 0·1 | — | — | 0·2 | — | — | 0·02 | 0·03 | + | — |
| + | — | 0·07 | 0·15 | 0·8 | — | ± | 0·05 | 1 | 0·002 | 0·02 | ± | +++ | — |
| (+) | — | 0·006 | 0·005 | 1 | — | — | 0·03 | 1 | — | — | 0·03 | +++ | — |
| ++ | — | 0·01 | 0·01 | 0·3 | — | — | 0·03 | — | — | — | — | — | — |
| (+) | — | — | 0·1 | 0·25 | — | — | 0·003 | — | — | — | 0·04 | — | — |
| — | — | — | 0·005 | — | — | — | — | — | — | — | — | — | — |
| c. 0·25 | 0·02 | 0·002 | 0·002 | 0·02 | 0·015 | 0·01 | 0·0005 | 0·2 | 0·001 | 0·01 | 0·003 | c. 1 | c. 0·5 |
| 0·002 | ? | ? | 0·000025 | < 0·00001 | ? | ? | 0·0001 | 0·00005 | ? | ? | < 0·003 | 0·0001 | 0·008 |

Some of the spaces for the gonad of *Pecten* are left blank. This is because part of the intestine and its contents were inadvertently removed with the gonad ; the contamination with detritus was therefore serious, and quantitative estimates for these elements would be both inaccurate and meaningless.

For further explanation, see text.

unable to reconcile this with my figures. It seems probable that lithium enters the organism along with sodium in a proportion determined by that existing in the medium, but that it is never specifically accumulated. It can apparently be laid down in an insoluble form in calcareous skeletons, for Vogel (1894) found it easily detectable in corals and mollusc shells, and Desgrez and Meunier (1927) in the teeth and bones of vertebrates. The latter claim to show that it is present as phosphate, but as the method involves solution of all the salts, followed by precipitation of the phosphates and detection of lithium in the precipitate, this can hardly be regarded as well founded. Robinson *et al.* (1917) find in a large series of plants a maximum lithium content of 0·00015 per cent.

Very little is known of the distribution of rubidium and cæsium in the organic or inorganic world. Sheldon and Ramage (1931) find that rubidium is constant in all human tissues, but its occurrence in lower animals and plants seems to be very sporadic. Clarke (1924) quotes analyses by Schmidt which reveal the presence of 10 to 15 mg. per litre of sea water, a value equivalent to about 3 per cent. of the potassium, but this has never been confirmed. The fact that Ramage finds the rubidium content of marine animals never to exceed 0·002 per cent. of the dry weight seems to render such a high figure unlikely. Elsewhere (1933, 1936) he finds much higher values in plants, up to 0·2 per cent. in *Cantharellus*.

Our knowledge of cæsium is even worse. Sonstadt (1870) claimed to detect it in sea water, algae, and mollusc shells, but Thompson and Robinson (1932) failed to find it in the concentrate from 200 kilograms. It seems to have been recorded once or twice in plants, but not in animals.

It is clearly not yet possible to draw any conclusions at all as to the biological significance of these metals. The matter possesses, however, some interest in that, among the very few experiments that have been carried out to determine whether one element can replace another in living tissues, those of Mitchell, Wilson and Stanton (1931) on the nutrition of rats, and Stanbury (1935) on the growth of diatoms showed that potassium can be to some extent successfully replaced by rubidium.

*The alkaline earths.*—The extraordinarily high magnesium content of *Littorina littorea* has already been pointed out by M'Cance and Shipp (1934). Table I shows that it is shared by *Nucella* and *Pleurobranchus*. The significance of this peculiarity is unknown. It is probably mainly in insoluble form, and may be stored in the liver, for Bialaszewicz and Kupfer (1936) have shown that the K : Na : Mg ratios for muscle are very constant for a wide series of marine and fresh-water forms of all phyla. The high magnesium content of the Echinoderms derives partly no doubt from their soft parts, but also from the skeleton which contains about 10 per cent. of magnesium carbonate (Clarke and Wheeler, 1922).

The remarkably high proportion of calcium in *Helix* may be ascribed to the deposits of calcium carbonate in the epithelium of the visceral hump, and of calcium phosphate in the liver (Sioli, 1935). It is clear that its calcium metabolism must differ greatly from that of the marine gastropods. The

remaining instances of a high calcium content are, except for the muscle of *Eupagurus,* provided by specimens that include calcareous skeletal matter.

Fox and Ramage (1931) were the first to show that strontium is generally distributed in marine animals. Table I confirms this finding, showing that the percentage of strontium varies from 2 per cent. of the cations in the mantle of *Archidoris* to a hundredth of this value, or less, in *Lineus, Ulva,* and some soft tissues of molluses. *Nephthys,* in which they failed to find any strontium, has a small but quite definite content of this element. It is curious that although strontium is the fifth most abundant metal in sea water, no estimate of its concentration was made until ten years ago. Desgrez and Meunier (1926) by a spectroscopic method arrived at a value of 13·5 mg. per litre. Their method, which assumes that the amount of strontium in solution after the sulphates have crystallised out may be neglected, seems rather precarious, and they assume a calcium content much greater than that usually accepted as normal for sea water. Ramage (1933) finds 40 to 50 mg. per litre, but Desgrez and Meunier's figure is confirmed by Thompson and Robinson (1932), and has therefore been adopted in the calculations necessary for correcting the standards for the strontium in sea water.

It is, therefore, not surprising to find considerable quantities of strontium in marine animals, and in the light of these figures the existence in certain Acantharia of a skeleton of $SrSO_4$ (Bütschli, 1906), which has usually been regarded somewhat as a minor miracle, is easier to understand. Strontium has often been recorded in plants, so that it is available also for terrestrial animals. But the supply is probably not so great as in sea water, and Ramage found it absent or very scanty in all the terrestrial forms examined with the exception of Pulmonates, particularly *Helix,* where its abundance is presumably connected with the large store of calcium.

It has been often asserted that barium is present in sea water, but no estimates of the concentration are available. According to Orton (1924) there is less than 200 mg. per cubic metre; but he gives no indication of how close to this maximum the actual value lies. The amount present in ionic form must be much less than this, for assuming the solubility of barium sulphate in water to be 2·3 mg. per litre, and the concentration of sulphate ions in sea water to be 2·7 g. per litre, the maximum concentration of barium ions possible in the sea is 0·48 mg. per cubic metre. (Thompson and Robinson seem to imply that ten times Orton's maximum value would be possible, but I am unable to see how this conclusion is arrived at.) Barium nodules are known from deep-sea deposits, but their origin seems to be as obscure as that of manganese nodules.

It will be seen that barium was found in about half the specimens examined, usually in quantities representing less than one ten-thousandth of the total metallic content. By far the highest concentration is in *Helix*; next come the mantle of *Pecten* and two of the algae. It is remarkable that it could not be detected in the shell of *Pecten,* but it is probably present in small quantities in the liver. This suggests the possibility that barium is absorbed along with

calcium and strontium, but a discriminatory power in the chemical processes of the secretion of the shell leads to the precipitation of barium in the mantle.

According to F. E. Schultze (cited in Samoilov, 1917) the skeleton of *Foraminifera Xenophyophora* consists of grains of barium sulphate. Ramage and Sheldon (1931) found barium in large quantities in the choroids of cattle, especially in old age. This would appear to be a specific idiosyncrasy, for the element could not be found in the choroids of a wide selection of other vertebrates, though most of them, particularly the marine fish, contained traces of strontium. Barium has several times been found in the ashes of terrestrial plants.

On the whole it would appear that there is more correlation of calcium with strontium in their biological distribution than of barium with either of the other two. Table I shows that the Ca/Ba ratio varies from 30 in the mantle of *Pecten* and 60 in Fucus to at least 30,000 in *Ophiocomina*, and perhaps more in those specimens in which barium cannot be detected. In *Helix* and the mantle of *Pecten* the Sr/Ba ratio is less than 1, but elsewhere it is over 250 and perhaps higher. On the other hand the extreme limits of the Ca/Sr ratio are 500 and 10, and in three-quarters of the specimens it lies between 250 and 30. The ratio in sea water is about 35, so that most marine animals seem to possess a definite, though limited, power of discrimination in favour of calcium. These ratios may be compared with those already published (Webb and Fearon, 1937) for the different regions of the Brazil nut. Ramage's (1936) figures for seeds also bear out the general correlation between calcium and strontium content.

As these three elements form a classical example of Döbereiner's triads, the properties of strontium and its compounds being intermediate in almost all respects between those of calcium and barium, it is difficult to suggest any purely chemical mechanism that would enable the organism to discriminate between strontium and barium more readily than between calcium and strontium.

*Boron.*—The quantity of boron in sea water is considerable, and it is easily detected spectroscopically. Moberg and Harding (1933) find the concentration fairly constant at about 4·5 mg. per litre; this corresponds to 0·03 per cent. of the cations. The borates must be considered as an important component of the buffer mechanism of sea water, being second only to the bicarbonates, and greatly outweighing the combined effects of phosphates, arsenates, and silicates.

Previous records of the biological distribution of boron are scanty. It has been found essential for the growth of plants (Warrington, 1926; Johnston and Dore, 1928), and is accumulated in relatively large quantities in soft fruits, up to 30 parts per million of the fresh weight (Dodd, 1929). Its constancy in animals is still dubious, and many mammalian tissues contain less than one part in a hundred million.

As may be seen from the table the concentration of boron is remarkably constant in marine tissues, and its relation to the total salt content is very close to that found in sea water. In three specimens it could not be detected, but as the limit of sensitivity of the method lies at a figure not far below many of the examples this may not be very significant. *Helix*, with a boron content of

about eight times the average, once more stands in sharp contrast to the marine forms. Of the latter, it may be said that their behaviour towards boron appears to be mainly passive, and that there is little or no tendency to accumulate the element or exclude it from their tissues.

Among the specimens examined, but not recorded in Table I, were some siliceous sponge spicules, prepared by boiling first in caustic potash and then in nitric acid, followed by repeated washing in distilled water, stray clay particles being eliminated by rapid decantation of the fluid. Two species were examined, both from Lough Ine, *Tethya aurantia* and *Suberites carnosus*. It was thought that traces of certain metallic silicates might be laid down with the silica of the spicule. Small quantities of calcium and magnesium were found in the spicules of *Suberites*, arising possibly from defective washing, and a trace of copper in those of *Tethya*; but the most remarkable finding was that in both cases the spectra showed strong indications of the presence of boron. It is impossible to give an estimate as to its quantity, for it appears that the emission of the boron lines is more favoured by a silica than by a sodium base, but it seems to be a constant and not inconsiderable minor constituent of the siliceous spicules.

*Aluminium and Silicon.*—These elements are grouped together—not because it is supposed that they present many similarities in their distribution or behaviour in living tissues, but because they present a common difficulty in the interpretation of analytical data. After oxygen, silicon is the most abundant element in the earth's crust, and aluminium comes next, so that all sand, mud. dust, or stray mineral matter of any kind is liable to contain large quantities of both elements, and it is exceedingly difficult to ensure that the external, digestive, and respiratory surfaces of animals are not contaminated with matter of this kind. In the rat, for example, which, living as it does under laboratory conditions, is far less exposed to mineral contamination than most littoral animals, M'Collum, Rask, and Becker (1928) found that the skin, lungs, and intestinal mucosa contained at least twice as much aluminium as any of the other organs. The only analyses of any worth are those of organs or tissues that do not include any such surfaces liable to contamination. Even when this precaution is taken, there always remains the possibility that some of the silicon may owe its origin to glass or silica apparatus.

The aluminium content of animal tissues has been the subject of some controversy. M'Collum *et al.* (1928) found by spectroscopic means that, except for the contaminated surfaces already mentioned, the organs of the rat, and also a large series of plant products, always contained less than 0·5 parts of aluminium per million of fresh weight. Kahlenberg and Closs (1929) claimed to show that large quantities were always present; but it is obvious, as M'Collum *et al.* (1929) point out, that they mistook the lines of calcium for those of aluminium. Subsequent authors, using both spectroscopic (Tourtelotte and Rask, 1931) and colorimetric methods (Myers and Mull, 1928; Myers and Morrison, 1928; Eveleth and Myers, 1936; Meunier, 1936), have made it possible to arrive at one part per million of fresh weight as about the average for

mammalian tissue, but the values for plants are still very varied and sometimes contradictory. From the results given here it would appear that the aluminium content of marine invertebrates is frequently less than 0·03 per cent. of the cations (in most cases corresponding to less than about 6 parts per million of fresh weight), but that in at least two cases where there is no reason to suppose that extraneous mineral matter can be present, the percentage rises to 0·08 (muscle of *Eupagurus* and ovary of *Paracentrotus*). Meunier's figures for the muscle of *Maia* and *Octopus* (8 and 11 parts, respectively, per million of dry tissue) are considerably less, so that on the whole it appears unlikely that the aluminium content of the tissues of lower animals is very much higher than in mammals.

Quantities of silicon of the order of 0·5 per cent. of the cations were found in these tissues of *Eupagurus* and *Paracentrotus*, and also in the mantle of *Pecten*; in all these instances the silicon may be presumed to be genuinely present in the tissue. This figure is no higher than that claimed by Kraut (1931) for human blood, 75 parts per million, which is equivalent to over 2 per cent. of the cations. Gonnermann (1920) found considerable quantities in the bile and urine, especially of Herbivora, in which it may give rise to siliceous vesical or biliary calculi. On the other hand, Isaacs (1924), using a method that was liable if anything to give high results, only found about 7 parts per million in mammalian organs.

The existence of siliceous skeletal elements in Radiolaria, Porifera, Diatoms, and certain higher plants, is well known. Two instances in Molluscs have been recently described (Labbé, 1933; Jones, M'Cance, and Shackleton, 1935) of skeletal structures that are wholly or partly siliceous.

The comparative scarcity of these two elements in living tissues in spite of their abundance in the inorganic world is partly explicable by their limited availability, which arises from the stability and insolubility of the compounds in which they occur. The silicate content of sea water is extremely variable (Atkins, 1928), and probably, as in the case of iron, the use of filters of different degrees of fineness gives very different results; Thompson and Robinson (1932) consider that the average range for shallow water is from 40 to 2,000 mg. Si per cubic metre. No quantitative data for aluminium are available, except for a provisional estimate tentatively advanced by Thompson and Robinson of 0·6 to 2·4 mg. per litre. It has long been known, however (Murray and Irvine, 1891), that diatoms are able to derive the silica necessary for their valves from suspended clay particles, so that the quantity of the element in solution may not be a true measure of its availability for organisms, and it is conceivable that small quantities of aluminium may be liberated in the gut of some animals from the decomposition of mineral particles.

In conclusion, it may be said, in so far as the analytical difficulties permit of the expression of an opinion, that while both these elements may appear as undoubted constituents of animal tissues there is, except for those forms with siliceous skeletons, only a small tendency to accumulate them from the environment.

*Vanadium.*—This element was detected only in two instances, *Pleurobranchus,* a tectibranch mollusc, and the pharynx of *Ascidiella.* The presence of vanadium in the form of a pigment in the blood of Tunicates, at any rate the Ascidiidae, has long been known (Henze, 1911), but it has never been previously recorded from a mollusc. Indeed the only other record of vanadium in the animal kingdom seems to be for *Stichopus möbii,* a Tortugas Holothurian, in which Phillips (1918) found 0·12 per cent. of the dry weight.

Vanadium is not known to occur in measurable quantities in sea water, though Thompson and Robinson (1932) were able to detect a "trace" in the concentrate from 200 litres. It is a constant minor constituent of most rocks, and constitutes on the average 0·035 per cent. of deep-sea deposits of the red clay type (Collett, 1932). Whether the animals can derive it directly from marine muds, or are forced to subsist on the infinitesimal quantities that are presumably present in solution, is unknown. In view of the very considerable amounts of vanadium found in some Ascidians the former would seem the more plausible explanation.

*Chromium and Molybdenum.*—Chromium was undoubtedly present in four of the specimens examined. It is just possible that in the case of *Ulva* it may be present wholly or partly in the form of mineral particles, since the values for aluminium and silicon are both high, but the fronds were perfectly clean to the naked eye, and it is by no means impossible that all three elements were actually present in the tissues. In the other three instances there can be little doubt that the chromium is of intrinsic origin, for it is absent from other forms from the same habitat which show at least as great a degree of mineral contamination. The quantity of chromium in sedimentary deposits seldom exceeds 0·1 per cent., so that, if the chromium in *Lineus* is to be explained as extraneous, the amount of mineral matter must be presumed to be at least as great as the amount of organic ash; since the silicon content is certainly not more than 2 per cent. of the total weight of metals, this is clearly impossible.

The results for molybdenum are much less certain, and have not been included in the table. The reasons for distrusting the figures for this element obtained by using graphite electrodes have already been explained. With silver electrodes the absolute arc sensitivity is considerably lower, and a concentration of 0·003 per cent. of the cations is on the border-line of visibility. In *Lineus* and the gonad of *Pecten* a doubtful trace, corresponding to about this concentration, could be seen. In *Littorina* and *Pleurobranchus* the indication was much more definite, particularly in the latter, and it is safe to suppose that in these animals the molybdenum represents about 0·006 per cent. of the cations. Certainly in no case does it rise to 0·01 per cent.

The status of chromium and molybdenum as biological elements has been hitherto rather dubious. Chromium has been detected spectroscopically in human tissues (Dutoit and Zbinden, 1930), milk (Zbinden, 1931), and marine animals (Newell and M'Collum, 1931), but all these findings relied on the use of graphite electrodes, and must be considered suspect. de Rubies and

Lemmel's (1935) record of the presence of chromium in the timber of 6 out of 26 species of trees is probably reliable, but it is purely qualitative.

ter Meulen (1931 and elsewhere) has claimed to show the presence of molybdenum in nearly all tissues, up to a maximum of 9 parts per million of dry weight in plants and 1·5 in animals. His technique has, however, been adversely criticised by Dingwall, M'Kibbin, and Beans (1934), who also gave reasons for supposing that the alleged concentration of molybdenum, as well as cobalt and nickel, in the nodules of leguminous plants (Forjaz, 1931) is without foundation. These authors, using a critical and reliable spectroscopic technique, found the molybdenum content of plants to vary entirely in accordance with the supply.

Neither element has been detected in sea water, and their concentrations must be exceedingly minute.

*Manganese.*—This element is now considered to be a general and perhaps universal constituent of all tissues, and the table shows that it was detected in all specimens examined except for the body fluids of the Echinoids. The variations in quantity are considerable, and not clearly correlated with any other factor. The value for *Helix* agrees well with that given by Boycott (1917); in most other Pulmonates the manganese content is far higher. Dubuisson and van Heuverzwyn (1930) find in most marine Gastropods 6 to 24 parts per million of fresh weight; the figures given here for *Nucella, Littorina,* and *Archidoris* probably fall within this range, but those for *Aeolidia* and *Pleurobranchus* are considerably lower. The incidence of manganese in molluscs is very irregular, rising as high as 315 parts per million of fresh weight in *Cerithium* (Dubuisson and van Heuverzwyn, 1930), and nearly 3 per cent. of the dry weight in certain slugs (Boycott, 1917). In some forms it is most abundant in the gills (van Heuverzwyn, 1930), in others the kidney, skin, or liver. In Crustacea it seems to be much more plentiful in the blood than in any of the tissues (Phillips, 1917).

Bertrand and Rosenblatt (1921) have shown that manganese is most abundant in those regions of plants in which the rate of metabolism is highest. This may account for the striking difference in manganese content between the receptacles of *Fucus* and the vegetative regions of the other two algae.

Thompson and Wilson (1935) have shown that sea water contains from 1 to 10 mg. of manganese per cubic metre. The concentration, like that of other minor constituents, probably varies in accordance with the rate of utilisation by the phytoplankton. The percentage in marine muds varies from 0·05 to 0·3.

It is clear that almost all organisms exhibit a tendency to accumulate manganese relative to the concentration in the environment, some of them to a remarkable extent. For a discussion of the various rôles in the organism that have been ascribed to this element reference may be made to Fox and Ramage (1931).

*Iron.*—It has long been realised that iron is a universal constituent of protoplasm, and additional interest has been recently given to the subject by

the respiratory theories of Warburg and Keilin. An extensive review by Schneider (1924) surveys the occurrence and possible function of this metal in animals. For reasons already explained, the method used here is only moderately sensitive for iron; nevertheless, the failure to detect it in two of the solid tissues as well as the Echinoid body fluids shows that in some cases its concentration is very low. The Gastropods are particularly rich in iron. In *Littorina* M'Cance and Shipp (1934) found it to be fairly evenly distributed through the tissues, but in *Patella* (Jones *et al.*, 1935), the head and mantle (not separated) and the liver contain four times as much as any other tissue. It is probable that in most cases iron is stored in the liver; its appearance in considerable quantity in the head of *Patella* is clearly connected with the observation, made by the same authors, that about 10 per cent. of the radula consists of iron.

The concentration is also very high in *Nephthys* and *Lineus*. The existence of hæmoglobin in the former may be a partial explanation, but the much lower figure for *Lepadogaster* shows that it cannot be a complete one.

Various estimates have been made of the concentration of iron in sea water. Cooper (1935) has made a careful study of the problem, and concludes that while the total quantity of iron in a cubic metre may be as high as 150 mg., it is doubtful whether there is ever more than 1 mg. of this in true solution. Much of it is in minute particulate form, both organic and mineral, small enough to pass all ordinary filters. It is shown on theoretical grounds that the concentration of ferric ions must be completely negligible, so that the fraction that does exist in solution is mainly ferrifluorides and perhaps some ferrous ions. The possibility that organisms can make use of the colloidal or particulate forms of iron must, of course, be borne in mind when considering these figures.

In view of Cooper's findings it is interesting to recall that Hopkins (1930) found 80 mg. per cubic metre the minimum amount of iron necessary to promote the growth of *Chlorella*, and that it was necessary for it to be in ionic form.

*Cobalt and Nickel.*—These closely related elements have been detected, by means of an exceedingly delicate chemical test, in almost all plant and animal tissues in quantities ranging from 0·002 to 2 parts per million of fresh weight (Bertrand and Macheboeuf, 1925; Bertrand and Mogkragnatz, 1925). These concentrations lie below the limit of sensitivity of all ordinary spectroscopic methods. Paulais (1936) found the nickel content of marine Lamellibranchs to vary from barely perceptible traces in the muscle to over 4 parts per million in the liver of *Cardium*. It appears, however, that some organisms contain much larger quantities; Fox and Ramage (1931) record 8 to 100 parts of nickel, and 20 to 60 of cobalt, per million of dry weight in a variety of polychaetes and molluscs. As a rule there is a strong correlation in the distribution of the two metals, though nickel tends to be more abundant and widely distributed, but they claim that the liver of *Archidoris* contains 30 parts per million of cobalt and no nickel. I am unable to find any cobalt, though I have examined two specimens, and the concentration falls above my limit of sensitivity. The

only specimens in which these elements were found were *Pleurobranchus,* in which there is a relatively enormous quantity of nickel accompanied by a smaller amount of cobalt, and three other cases in which the amount of nickel lies near to the limit of visibility. It must, therefore, still be considered doubtful whether organisms possess the power of discriminating between these two elements, though the tendency on the part of certain species to accumulate them seems well established.

Neither element has been detected from sea water in spite of the very delicate tests that are available.

*Copper.*—An enormous literature relating to the distribution of copper in living tissues has sprung up in the last few years, and it is impossible to attempt here even to survey the field. In the form of hæmocyanin it discharges a respiratory function in certain Crustacea, Arachnids, and Molluscs, but the precise distribution of hæmocyanin within these groups is not yet at all adequately known. In mammals it is necessary for the synthesis of hæmoglobin (M'Hargue *et al.,* 1928; Hart *et al.,* 1928). In the feathers of the turaco it is present as the salt of uroporphyrin (Fischer and Hilger, 1924), and it is reasonable to suppose that it plays an important part there as a detoxicating agent. Beyond these instances, little is known of the physiological significance of copper; it may act as a catalyst for intracellular oxidations, and it appears to be component of all living tissues. From an examination of the data of Cunningham (1931), Phillips (1917 and 1918), M'Hargue (1924), and Jones *et al.* (1935), it would appear that most marine invertebrates that do not contain hæmocyanin have a copper content of 10 to 50 parts per million of dry weight. In all forms, whether they contain hæmocyanin or not, the liver tends to be particularly rich in copper, as in many other metals. This applies also to vertebrates, and Ramage, Sheldon, and Sheldon (1933) have shown that large quantities are stored in the liver of the fœtus. Oysters, although they do not contain hæmocyanin, possess a very high copper content, rising in the case of "green" oysters to about 3,000 parts per million of dry weight (Orton, 1924; Galtsoff and Whipple, 1931).

The results in the table show that only those species in which hæmocyanin is known to be present have a copper content of more than 0·1 per cent. of the cations. It is clear that none of the Opisthobranchs or Lamellibranchs examined can possess hæmocyanin. Svedberg and Hedenius (1934) have already shown that it is absent from all Lamellibranchs so far examined (previous statements to the contrary notwithstanding), and also from *Aeolidia, Dendronotus,* and *Patella.* It seems, therefore, highly probable that the distribution of hæmocyanin in the Gastropods is not so wide as has been hitherto assumed, and that it is unknown in the Opisthobranchiata.[7]

The variation of copper content in the remaining species does not appear to have any obvious significance. The most remarkable finding is the apparent

[7] The only thoroughly reliable records are for a dozen or so genera distributed between the Pulmonata and Pectinibranchiata.

absence of the element from the stipe of *Saccorhiza.* A careful search with more refined methods would doubtless demonstrate its presence in minute quantities, but it is clear that its concentration relative to the total weight of cations is little, if at all, greater than in sea water.

The amount of copper in sea water has until lately been taken as 100 to 200 mg. per cubic metre, but a recent careful determination by Atkins (1932) has shown that 10 mg. per cubic metre is the correct figure.

*Silver.*—Fox and Ramage (1931) found that silver had a wide but very spasmodic distribution. In the absence of the necessary quantitative data it is impossible to say to what extent this may be due to random distribution of values around the limit of sensitivity. They found 10 parts per million of dry weight in the liver of *Helix,* and quantities of 50 parts and over in the livers of marine lamellibranchs. Ramage (1930) found 2,000 parts per million in Fungi. There are few other reliable records, and none of them are quantitative.

The data for silver in this series of analyses are incomplete, for reasons already given. It was detected in about half the samples examined, but the maximum concentration observed was about 0·003 per cent. of the cations. This value was recorded for *Fucus,* and agrees well with Malaguti, Durocher, and Sarzeaud (1850), who found 10 parts per million of ash in the same material. The concentration of silver in sea water has been variously estimated, but Thompson and Robinson give 20 mg. per cubic metre as the most probable value. It would appear, therefore, that in the species examined the concentration does not exceed 25 times that which obtains in the external medium.

*Zinc.*—The difficulties involved in the estimation of this element, and the reasons for the low degree of sensitivity of the method, have been already described. The results are nevertheless very interesting. Of the six species of Gastropods three contain large quantities of zinc, and the other three little or none. The liver, gonad, and mantle of *Pecten* are all rich in zinc, but it cannot be detected in the gonad of *Modiolus,* which must, therefore, contain less than a tenth of the quantity found in the gonad of *Pecten.* It is plentiful in *Lineus* and *Nephthys,* and there is probably a trace in *Lepadogaster,* but none of the other specimens give evidence of its presence.

Dieulafait found 2 mg. of zinc per cubic metre of sea water, Bodansky (1920) 7·3 mg. (This latter figure has been several times misquoted as 73 mg.) Probably the true value lies between. Atkins (1936) has confirmed that there is less than 8 mg. It seems, therefore, that some animals are able to accumulate zinc up to a concentration of 20,000 times that in the external medium.

Among biological metals zinc is second only to copper in the attention that has been paid to it in the last few years. Zinc contents of upwards of 100 parts per million of fresh weight or 500 parts per million of dry weight have been recorded for the fowl (Bertrand and Vladesco, 1921), the viscera of *Limulus* (Phillips, 1917), lamellibranchs, especially oysters (Orton, 1924; Bodansky, 1920; M'Hargue, 1924), and a single species of fish (Bodansky, 1920). In most plants and in various other species of animals zinc, while usually detectable, is much less plentiful. The variation among different species

of gastropods shown in the table is very striking; Phillips (1917) also found that *Fulgur* contained six times as much zinc as *Strombus* or *Aplysia*. The zinc content does not seem to be correlated with diet, taxonomic position, or even the presence of hæmocyanin. The existence of a pigment "hæmosycotypin" in the blood of *Busycon* (= *Sycotypus*), supposed by Mendel and Bradley (1907) to contain zinc as well as copper, has recently been disproved by Gatterer and Philippi (1933).

Various pieces of evidence have been brought forward at different times to show that zinc is of physiological importance in the organism.

(1) Stirn, Elvehjem, and Hart (1935), and Bertrand and Bhattacherjee (1935) have shown that rodents fed on a diet deficient in zinc, though adequate in other respects, do not grow to maturity.

(2) It has been shown that, if traces of zinc are present, the hypoglycæmic action of protamine-insulin or spermine-insulin compounds (which are more potent than pure insulin) is greatly prolonged (Fisher and Scott, 1936; Biasotti *et al.*, 1936).

(3) Bertrand and Vladesco (1921 *a*) have shown that the accessory glands of the male genital apparatus in mammals are rich in zinc, and the seminal fluid even richer, and suggest that the metal may play a part in fertilisation.

(4) Delezenne (1919) held that the power of snake venom to bring about rapid hydrolysis of nucleoproteins and phosphatides was correlated with its large zinc content. Cristol (1923) was led to conclude that this property of zinc enabled it to act as a necessary catalytic agent for the chemical changes that take place at mitosis. He observed that in malignant tumours or in the blood and liver in cases of leucocythæmia—both tissues in which mitosis is proceeding very rapidly—the zinc content was from 3 to 18 times the normal.

*Cadmium.*—The only previous records for cadmium in living tissues are for the liver of *Pecten* (Fox and Ramage, 1931), and traces in the liver of sheep and salmon (Ramage, 1936 *a*). The table shows that small quantities of the element are not uncommon in molluscs and echinoderms. The liver of *Pecten* was examined, and found to contain cadmium, in concentration probably somewhat greater than any of the gastropods, but not sufficiently high for it to be regarded as a specific idiosyncrasy.

Cadmium has never been recorded from sea water. It is probable that it has been included in all estimates made by chemical means of the zinc in sea water or tissues.

It will be noticed that high concentrations of cadmium are not always accompanied by high concentrations of zinc, and the degree of correlation between the elements seems to be fairly small.

*Tin.*—The only reliable data on the occurrence of tin in organisms are the analyses of Orton (1924), who found from 10 to 55 parts per million of fresh weight in oysters (the higher figure only for green specimens), and of Bertrand and Ciurea (1931), who found 0·4 to 4 parts in most mammalian organs, but up to 26 in the lingual mucosa. Misk (1923) had previously published much

higher figures, but according to Bertrand and Ciurea his method is one that estimates silica as well as tin.

Its distribution in marine invertebrates seems to be very sporadic. Only the figures for *Aeolidia* and the two echinoderm samples are really significant; the others are only just above the limit of visibility, and may be nearly approached by some specimens in which no tin can be detected.

Tin has never been detected in sea water.

*Lead.*—This metal seems to be more widely distributed in detectable quantities than tin, but its occurrence is just as sporadic and unpredictable. This confirms the general conclusions of Fox and Ramage. The highest concentration was found in *Helix*; but according to these authors it is not invariable in this animal, and may not be detectable in individuals from some localities. The high figure for *Fucus*, in contrast to the other algae, is striking.

Although the distribution of lead in the human body has been intensively studied from the point of view of industrial hygiene and occupational disease, our knowledge of its distribution in lower organisms is rather limited. Phillips (1917) found only 2 parts per million in the liver of *Fasciolaria*, and none in a number of other marine forms. Bishop (1928–9) claimed to find up to 10 parts per million in the yolk of the hen's egg, mainly in the form of lead oleate, whereas Taylor (1930) found less than 0·1 part per million. Each author criticised the methods of the other, and the controversy has never been satisfactorily settled. Chapman (1926) found that various edible molluscs and crustacea contained from 5 to 26 parts per million; this corresponds to the range of the majority of the figures in the table. Oysters are able to accumulate large quantities of lead as well as copper, zinc, and tin, and may contain as much as 400 parts per million fresh weight.

The only estimate of lead in sea water appears to be that of Chapman, who finds 400 mg. per cubic metre. This badly needs confirmation, and it seems probable that the true value may be considerably lower.

*Phosphorus.*—The results for this element are only incidental, and have not been worked out quantitatively. As might be expected, the reproductive tissues and the fish (on account of its phosphatic skeleton) are the richest in phosphorus.

The limit of sensitivity is about 1 per cent. of the cations.

*Fluorine.*—Fox and Ramage found fluorine to be present in the mantle of *Archidoris*, and this finding is confirmed. It is, however, present also in other parts of the body. The quantity is very great; certainly equivalent to 2 per cent. of the cations, and perhaps more. It must not be assumed that it is necessarily present as calcium fluoride. A smaller but quite definite quantity was found in *Ophiocomina*, and a somewhat doubtful indication in *Marthasterias*.

Fluorine is known to constitute about 1 per cent. of the dental enamel,[8] and

[8] While this was in the Press a paper by J. H. Bowes and M. M. Murray (*Biochem. J.*, **29**, 102; 1935) has come to my notice, in which it is shown that in both dentine and enamel there is only about 0·03 per cent. of fluorine.

to occur in smaller quantities in bones, but otherwise it seldom exceeds 5 parts per million in animal material, though it is often more plentiful in plants. It is not known whether it is laid down in the calcareous skeletons of invertebrates to the same extent as in bone; Carles (1907) found 120 parts per million in oyster shells, but his method, which relied on comparison of the intensities of the etching of glass, was rather precarious, and led to a wrong value for sea water.

The latest estimate of the concentration of fluorine in sea water is 1·4 mg. per litre (Thompson and Taylor, 1933). Previous determinations have usually been somewhat lower.

## VI.—Conclusion.

Throughout the foregoing discussion considerable stress has been laid on the concentration in which each element exists in the environment, and also on the limit of sensitivity of the method for the different elements treated of, for it is clear that unless these considerations are constantly kept in mind the words "present" and "absent" as applied to elements in living tissues are almost meaningless. In particular, it is important to remember that the limit of sensitivity, whether chemical or spectroscopic tests are used, varies from one element to another in a completely arbitrary and fortuitous fashion, and that it is, therefore, quite possible that zinc, for example, has just as universal a distribution in biological material as has copper, and that apparently sporadic distributions may arise solely from failure to detect very small concentrations. Constancy of the presence, within the limits of sensitivity of any particular method, of an element throughout a wide range of species cannot accordingly be considered a reliable criterion of physiological importance, since it is possible either for it to be stored up, if the available supply is particularly abundant, in quantities considerably greater than those necessary for the discharge of the physiological function; or, on the other hand, for the element to perform two functions, one general throughout the animal kingdom, and one specific, within a small group. This would appear to be so with copper.

In spite of these difficulties it is plain that considerable differences exist within the group of biological elements according to the degree of variation in their concentration from one organism to another, and from organism to environment. The data are not yet sufficient for generalisations to be anything but hazardous, but it seems likely that a division of the biological elements into four groups is possible, as follows:—

(1) The architectural elements, whose abundance in the environment has clearly made them one of the primary conditions of life, and which, even if they are less abundant in the organism than in the environment, are clearly of fundamental importance and significance in the life of the organism. This group includes Na, K, Mg, Ca, and also the non-metals, such as C, H, O, N, S, and P, which have not been treated of here.

(2) Those elements which can always be detected by sufficiently refined methods in the tissues of organisms, but in concentrations so small that the rôle of the organism may be supposed to be entirely passive. They are never accumulated to any great extent. As far as marine animals are concerned, Li, Sr, B, and Al belong here, with Ag probably intermediate between this and the next group.

(3) Those elements which are nearly always found in the organism in concentrations considerably higher than those that obtain in the environment, but which, compared with the elements of the next group, are relatively constant from one organism to another, are never accumulated in really large quantities, and give no evidence for supposing that they are of specific importance to any particular group. Pb, Ba, and Mn may be tentatively assigned positions here if only the organisms whose analyses are here published are considered; but it must be remembered that Mn occurs in very large quantities in certain land and fresh-water molluscs.

(4) Those that occur in remarkably high concentrations in certain groups or species, and for whose physiological importance within these groups or species there is an *a priori* case. In some cases the element may also occur in smaller, but still significant, concentrations in the majority of other organisms, as in the case for Si, Cu, Fe, and perhaps Co and Ni. In others this is unlikely or still uncertain—e.g., V, Cr, Zn, Cd, Sn, and F.

It is impossible from as small a number of analyses as this to draw any reliable conclusions as to the relative amount of variation in inorganic constituents between the same organ in different species, and between different organs in the same individual. It is clear, however, that certain organs such as the liver (using the term in a loose sense) have a tendency towards a high metal content in all groups. The question of the causes of variation between different species of the same taxonomic group is almost equally obscure, but a study of the data here presented for Gastropods would suggest that diet is probably more important than habitat, as far as the minor constituents are concerned. *Littorina* (vegetarian, littoral) exhibits almost as many differences in composition from *Nucella* (carnivorous, littoral) as from *Helix* (vegetarian, terrestrial). *Aeolidia* and *Pleurobranchus* are found in very much the same habitat and have the same general *modus vivendi*. *Aeolidia* feeds entirely on sea-anemones; there seems to be no record of the food of *Pleurobranchus*, but it is possible that it might browse on Ascidians and retain in its tissues the vanadium thus obtained. Further corroboration is given for this view by analyses of *Littorina littoralis* and *L. rudis*. These species are also vegetarian, but live higher up the shore than *L. littorea*. Owing to their small size, and consequent high ratio of surface to volume, the degree of mineral contamination was too great for their analyses to be set out in the table, but it was easy to see that in their general composition, especially the presence of considerable quantities of lead, and in the absence of barium, cadmium, or any appreciable amount of zinc, they approximated closely to *L. littorea*.

Elements present in the tissues of animals may be derived either from food (ultimately plants) or water. For terrestrial animals the only water is that

which they drink: for aquatic animals it includes the water that bathes their external surface as well as any that they may swallow. The data are rather scanty, but it appears that terrestrial plants are at least as rich in most micro-constituents as are terrestrial animals, so that the former may be considered to be the primary concentrating agent, and the animals are able to make use of their activity. It was hoped that these analyses might show whether the same held true for the food cycle in the sea, but the three samples of plant material are of such widely different composition with regard to micro-constituents that it is impossible to reach any definite conclusion. In any case it would be essential to take account of the phytoplankton as well as the sessile algae. The extreme poverty of the stipe of *Saccorhiza* in all but the primary elements is perhaps exceptional; but it may be recalled that one animal, at any rate (*Patina pellucida*), lives solely on such material, and must, therefore, possess the power of concentrating those elements it requires direct from the sea water. And though no doubt the analyses of *Ulva* and the receptacles of *Fucus* are more typical of algal material that contains appreciable amounts of photosynthetic or reproductive tissues, it would seem probable that in respect of the necessary elements the dependence on plants is less in the sea than on land.

## VII.—Summary.

1. A number of animal and plant tissues, mainly of marine invertebrates, have been subjected to spectrographic analysis, and data for 25 elements are presented.

2. The results are mainly quantitative, and special attention has been paid to the concentration of the elements in sea water and to the limits of sensitivity of the method for each element.

3. Lithium, boron, strontium, aluminium, and silver were frequently detected, but never in quantities that represented a significant accumulation of the element from the environment.

4. Barium and lead have a spasmodic distribution.

5. Vanadium, nickel, and cobalt were found in relatively large quantities in *Pleurobranchus* (a tectibranch mollusc). Smaller quantities of nickel were found in three other species.

6. Chromium was found in *Lineus*.

7. Manganese was found in almost all cases, but nowhere very abundant.

8. Iron and copper are very general, but the quantity is very variable. In the case of copper this is dependent on the presence or absence of hæmocyanin. It is suggested that this pigment is absent from all Opisthobranchs.

9. Zinc is present in very large quantities in some species, but could not be found in others closely related. Cadmium was frequently detected.

10. Tin is abundant in *Aeolidia* and two Echinoderms.

11. The presence of fluorine in the mantle of *Archidoris* is confirmed, and the quantity is found to be considerable. It is also present in other parts of the body and in *Ophiocomina*.

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